

ULTRASTRUCTURAL CHANGES IN
THE CILIARY EPITHELIUM FOLLOWING
INHIBITION OF SECRETION
OF AQUEOUS HUMOUR IN
THE RABBIT EYE

An experimental study by means of electron microscopy

BY
ÅKE HOLMBERG

Diss. med.
Stockholm. Karolinska Institutet
17 APR. 1957

STOCKHOLM 1957

FROM THE LABORATORY FOR BIOLOGICAL ULTRASTRUCTURE RESEARCH OF
THE DEPARTMENT OF ANATOMY, KAROLINSKA INSTITUTET, STOCKHOLM,
AND THE OPHTHALMIC CLINIC, SABBATSBERG HOSPITAL, STOCKHOLM

ULTRASTRUCTURAL CHANGES IN
THE CILIARY EPITHELIUM FOLLOWING
INHIBITION OF SECRETION
OF AQUEOUS HUMOUR IN
THE RABBIT EYE

An experimental study by means of electron microscopy

BY
ÅKE HOLMBERG

STOCKHOLM 1957



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA40153502_0069

To my wife

Printed by
AXLINGS BOK- & TIDSKRIFTSTRYCKERI
Södertälje, Sweden 1957

CONTENTS

Preface	7
General introduction	9
Chap. I. Material and methods	10
Chap. II. The normal structure of the non-pigmented ciliary epithelium	12
Earlier observations	12
Results	13
β -cytomembranes	13
α -cytomembranes	16
Golgi apparatus	18
Cell membrane and intercellular border	19
Terminal bars	20
Basement membrane	21
Mitochondria	22
Nucleus	24
Large granules	25
Large spherical bodies	26
The ground substance of the cytoplasm	26
Discussion	27
Chap. III. Changes in the structure after injection of Diamox	40
Introduction	40
Material and methods	40
Results	42
Functional changes	42
Ultrastructural changes	43
Mitochondria	44
Golgi apparatus	46
Cytoplasm	52
Discussion	57
General discussion	62
Summary	66
References	69

PREFACE

This study has been carried out in the Laboratory for Biological Ultrastructure Research of the Department of Anatomy, Karolinska Institutet, Stockholm, and in the Ophthalmic Clinic, Sabbatsberg Hospital, Stockholm.

The head of the Laboratory, Doctor Fritiof Sjöstrand, Associate Professor of Anatomy, has facilitated the work in every way and followed it with the greatest interest. He has given many useful suggestions with regard to the presentation of the material.

The resources of the Department of Anatomy were placed at my disposal through the kindness of the head of the department, Professor Ture Petrén.

The head of the Ophthalmic Clinic, my teacher in ophthalmology, Docent Ingemar Kugelberg, M. D., has followed my investigations with the greatest interest and facilitated my work in different ways.

Docent Hans Engström called my attention to ultrastructural research.

Professor Ernst Bárány, Docent Arne Huggert and Doctor Per Wistrand have given much valuable advice in discussion of the experiments with Diamox.

Fil. lic. Ulf Strömberg has given valuable advice and great help with the statistical evaluation of the material.

Miss Barbro Runling has been of greatest help to me through her technical assistance. She has printed all the pictures and she has drawn all the diagrams.

Miss Maj Berghman has done the schematic drawings.

The Swedish manuscript was translated into English by Mr Cyril Marshall, and help with the technical terms has been given by Professor Vincent Hall.

To all these persons I wish to express my most sincere thanks.

The investigation has been rendered possible by support from Stiftelsen Therese och Johan Anderssons Minne and from the Swedish State grants for supporting medical research.

Stockholm, March 1957.

Ake Holmberg

GENERAL INTRODUCTION

In recent years investigations of the dynamics of the aqueous humour have shown that the ciliary epithelium plays a significant rôle in the mechanism lying behind the production of the aqueous humour. Study of the rate of passage of the different substances from the blood to the posterior eye chamber has led to the assumption that the transport probably takes place through a membrane system with a selective ability to arrest or facilitate it (Davson *et al.*, 1949). Other investigations (Bárány, 1947 a, b, c., Kinsey and Bárány, 1949, Duke-Elder and Davson, 1949, Ross, 1951, and others) show that the ciliary epithelium participates actively in the production of the aqueous humour, which is therefore secreted, at least in part.

The purpose of the present investigation was to analyse with the aid of electron microscopy the finer structure in the ciliary epithelium and clarify the structural organisation which constitutes the basis for the activity of the cells. The investigation comprises two sections. The first consists of a study of the structure of the epithelium in normal conditions. In the second an attempt has been made to correlate the ultrastructure with experimentally induced changes in the formation of aqueous humour. Such a change can be obtained by supplying Diamox, a strong inhibitor of carbonic anhydrase. As will be touched on later, Diamox causes an inhibition of aqueous humour production by some 60 %. When the investigation was started it was the intention also to study changes after increased aqueous humour production but, unfortunately, no method was found which could definitely provide an increase in that part of the aqueous humour which is secreted.

CHAPTER I

MATERIAL AND METHODS

The experimental animals employed were rabbits weighing between 2 and 3,5 kg. These included both albinos and pigmented animals. The distribution in respect of sex was approximately equal.

The material was fixed in 1 % osmium tetroxide solution in a veronal acetate buffer (Palade, 1952 a) modified according to Sjöstrand (1953 d).

It was found at a fairly early stage that great difficulty was involved in obtaining a satisfactory fixation, free from post mortem changes, by using the routine method of cutting out a piece of corpus ciliare and then fixing it. Consequently, in all the material here presented the fixation was all started *in vivo*. The animals were anesthetized with nembutal 0.6 cc per kg body weight of a 10 % solution given intravenously. At the site of corpus ciliare about 0.5 cc osmium solution was injected by diascleral puncture. It was deposited as near the epithelium as possible to avoid too much of the injection getting into the vitreous body. At the same time as the injection, the aqueous humour was tapped by paracentesis at limbus corneae. After fixing for 15—30 min., parts of corpus ciliare were extirpated and fixing then proceeded in the usual manner. In the albinos, it was very easy to localise those parts which had been in contact with the fixing solution.

The total fixation time in osmium solution varied between half an hour and 4 hours. Though no systematic investigation was made it appears that a fixation time of between 3 and 4 hours gave the best results. In material fixed in shorter times, it seemed that considerable artefacts arose owing to material being released in the course of the dehydration following the fixation. Nevertheless, such cell constituents as cytoplasmic membranes, nuclei, mitochondria etc. appeared to be well preserved even after short periods of fixation.

Following the osmium fixation the tissues were treated as follows:

Tyrode's solution	1/2 hr.
70 % alcohol + 0.1 % phosphotungstic acid	1 hr.
95 % alcohol	1 hr.
abs. alcohol	1 hr.
methacrylate mixture	1 hr.

Embedding was done according to the method of Newman, Borysko, and Swerdlow (1949). A mixture of 85—90 % butylmethacrylate and 10—15 %

methylmethacrylate was used with the addition of 0.1—0.5 % benzoyl peroxide. Pre-polymerisation invariably preceded the final polymerisation, which was done at 40—45° C for 24—36 hours up to required degree of hardness.

Sectioning was performed on the Sjöstrand Ultramicrotome (Sjöstrand 1953 b) using both specially polished razor blades (Sjöstrand, 1953 b) and glass knives. In trimming the specimen for sectioning only the epithelium in the middle part of corona ciliaris was taken. Thus, the posterior parts next to ora serrata and the anterior parts next to iris are not included in this investigation.

The examination of the sections was done in a RCA EMU-2c electron microscope with compensated objective lens pole piece, with an aperture in the objective lens of 50 μ , with three apertures in the projector lens and reduced condensor aperture.

The determination of the finer dimensions was made on photos taken with at least 8,500 times electron optical magnification and afterwards magnified 5 times.

The fixation method applied appears to be particularly well suited to material of the kind here used. Owing to the fixation liquid diffusing into the cell from one direction and the blood supply to the cell coming from the opposite direction, death of the cell takes place as and when fixation starts. Thus, there is minimum time for post mortem processes to take place. In the usual method of fixation, the animal is first killed and the required tissue then removed. When this method is applied to the corpus ciliare, part of the vitreous body accompanies the specimen and forms a protective envelope over the epithelial cells, which keeps the fixative from reaching the cells until after post mortem changes similar to those described by Rhodin (1954) and Zetterqvist (1956) were developed.

Although, regarding the role of the phosphotungstic acid in the fixation process, no systematic investigation was made, it was employed in the earlier investigations for the purpose of improving the contrast in the specimen and was used throughout in the whole of the investigation for the sake of continuity. There is no doubt that the phosphotungstic acid increases the contrast appreciably, even in the small concentrations here used. For the staining of muscle cells for electron microscopy concentrations of phosphotungstic acid of between 0.5 and 1.0 % are used as a rule.

CHAPTER II.

THE NORMAL STRUCTURE OF THE NON-PIGMENTED CILIARY EPITHELIUM

EARLIER OBSERVATIONS

At the ora serrata the retina changes over to a single layer of epithelium which forms the inner layer of the double epithelial cell layer that covers corpus ciliare. The inner layer is non-pigmented, the outer pigmented. Next to ora serrata, within orbiculus ciliaris, the inner layer is built up of cylindrical cells, comparatively low to start with, whereas further on towards corona ciliaris the cell height increases. Cell heights between 40 and 50 μ have been measured in this region (Fuchs, 1917). Within corona ciliaris the cells become lower, mostly cubical, to become still lower in the region immediately behind iris while at the same time pigment begins to appear in this layer also.

A fixed connection by means of a cement substance exists between the cells and this is particularly firm between the two layers (Lauber, 1931). It has, in fact, proved difficult to separate the non-pigmented layer from the pigmented (Lauber, 1931).

The cells are covered by a cuticular layer which some investigators (Czermak, 1885, and others) consider to be a direct continuance of the internal limiting membrane whereas others (Salzmann, 1912, Lauber, 1931) consider it to be a separate membrane which ends at ora serrata. The membrane is firmly attached to the cells and often forms a process between them (Lauber, 1931, and others). This membrane is considered by many researchers to be an anchorage point for zonule of Zinn (Czermak, 1885, Salzmann, 1912, Lauber, 1931, and others).

Mawas (1910) and others have described the nuclei of the non-pigmented epithelium as round or oval, often furnished with incisura and located in the basal part of the cell. It displays a pronounced polychromasia (Mawas, 1910). v. Ebner (1899) demonstrated for the first time fibrous structure in these cells. The fibres were orientated mostly in the long direction of the cells and above all localised close to the nucleus. In later investigations (Mawas, 1910, Seidel, 1920, Gilbert, 1921, and others) it has been shown under high magnification that these threads were made up of rods or grains and they were considered identical with mitochondria. Mawas (1910) was able to show in unfixed material that the cells were filled with strongly refracting small grains and, therefore, the structures in the form of grains or rods which were

observed on fixed material were assumed to be pre-formed formations. In the peripheral parts and particularly at the surface of the cells Seidel (1926) among others observed numerous vacuoles. The same author has also shown that in those cells where vacuoles occurred numerous the mitochondria were few.

No detailed analysis of the finer structures in the ciliary epithelium has been made previously with the electron microscope although Holmberg (1955) and Pease (1956) reported that these cells display many elaborate folds by which a system of double membranes in the cytoplasm arises. According to Pease these folds reach deep down in the cell, in some cases they would extend through practically the whole cell.

RESULTS

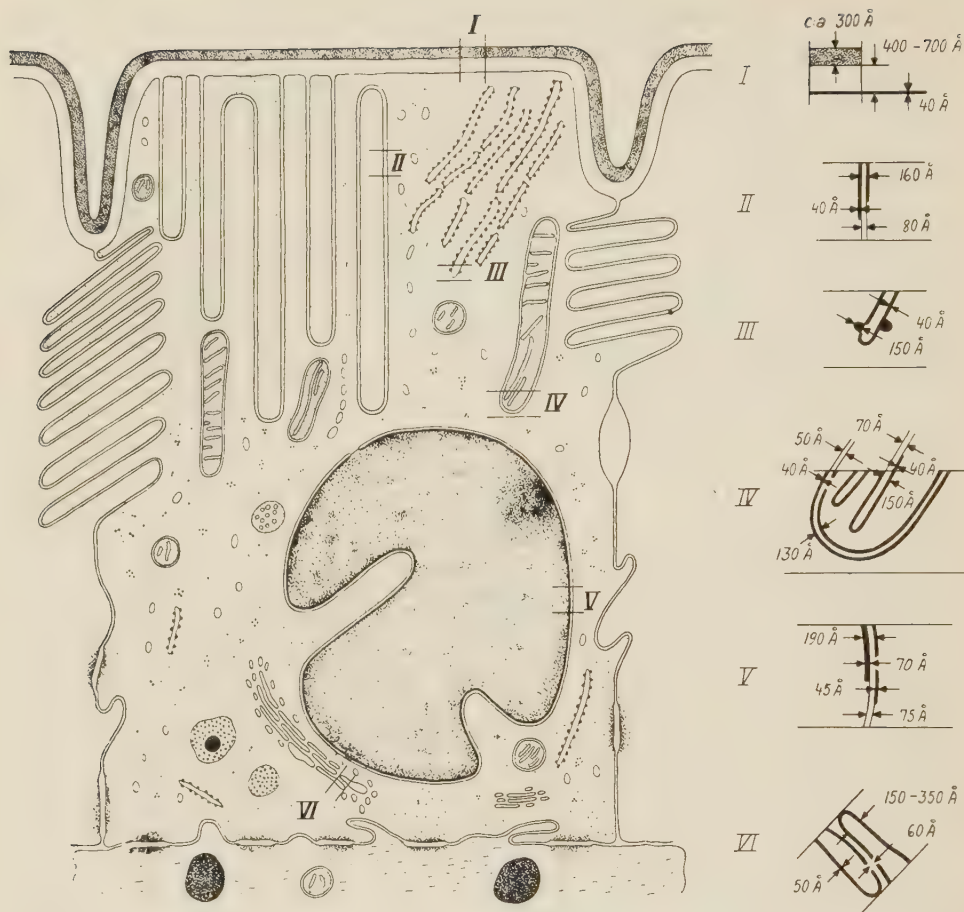
β -cytomembranes

Sjöstrand (1956 c) defines the β -cytomembranes as "membranes which represent tight folds of the cell membrane extending into the interior of the cell body and which do not constitute boundaries facing the surrounding milieu, that is, the two membranes of a fold are in close contact".

β -cytomembranes constitute a characteristic component of the non-pigmented ciliary epithelium. The membranes are entirely located in the upper quarter of the cell and are never found lower than the level of the upper part of the nucleus (Fig. 1). The extension of the membranes in this direction varies between 1.5 and 2.5 μ . The extension of the membranes in the other direction is rather uncertain owing to the difficulty in obtaining exact tangential sections. Nevertheless, they do not extend through the whole cell. This is evidenced by the fact that some of the sections entirely lack membranes and on others isolated membranes may be seen inside the cytoplasm without connection to the cell surface (Fig. 7). The measurements taken of the extension of the membranes in this direction give values between 2 and 3 μ .

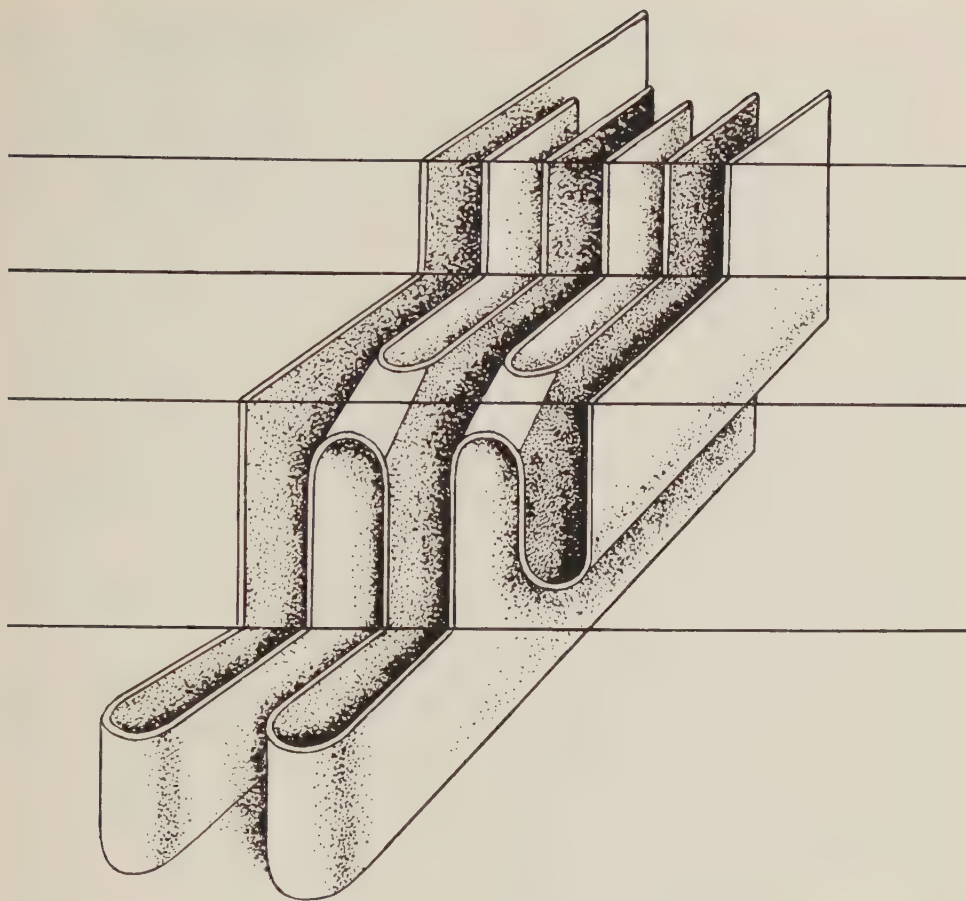
One consequence of the limited extension of the membranes in the transverse axis of the cell is that the number of membranes in the different sections varies considerably (Figs. 2 and 3). The highest number of double membranes observed in a cell is 62. The mean number in 27 cells counted amounts to 34.

The membranes in the ciliary epithelium are very irregularly distributed in the cell. In most cases they are grouped around the indentation between the cells (Fig. 1), which is so common, but they may occur anywhere in the upper layer of the cell.



Textfig. 1. Schematic drawing of non-pigmented epithelial cell in the ciliary body. To the right the dimensions are given for (I) the basement membrane and the cell membrane, (II) the β -cytomembranes, (III) the α -cytomembranes, (IV) the mitochondrial membranes, (V) the nuclear membrane and (VI) the γ -cytomembranes.

The membranes occur without exception as double membranes, i. e., they are made up of two opaque layers which surround a less opaque middle layer. On some pictures it has been observed that the opaque layers consist of three layers (Fig. 6). The membranes run usually more or less at right angles to the cell surface but here and there they may be seen oriented almost parallel with it. They have a very characteristic arrangement as in most sections they form U-shaped loops (Figs. 5 and 6) in their longitudinal section with both branches connecting with the cell surface. In other sections the membranes



Textfig. 2. *Three-dimensional picture of the β -cytomembranes.*

appear as isolated closed loops (Fig. 7) and in yet another sections they are irregularly arranged in long winding loops. From these different appearances in sections it has been possible to propose a tree-dimensional reconstruction of the membranes (Textfig. 2).

Now and then anastomosis may be seen between adjoining membranes. Such anastomosis is arranged in such a way that the middle layer in a membrane is in communication by way of a transverse membrane with the middle layer in an adjacent membrane (Fig. 7).

The membranes are for the most part very regularly built up, in so far as the separate components lie at a comparatively constant distance from each

other (see below for quantitative data). Here and there, however, the membranes expanded so that vacuoles arise (Figs. 4, 5 and 7) which may assume quite large dimensions. The largest vacuoles measure between 0.2 and 0.6 μ . In most cases, two or more membranes form these vacuoles. In no case has any content been observed in the vacuoles. They display the same density as the embedding material itself. Except for what has been stated earlier, no definite relation between β -cytomembranes and other cell parts has been observed.

As regards quantitative data for β -cytomembranes, the following additional particulars may be given. Altogether 30 membranes were measured representing 19 cells from 9 animals. About 15 measurements were made on each membrane. The mean of these has been taken for computing the mean for all membranes. The total thickness of the membranes is $160 \pm 3 \text{ \AA}$ (SD = 15, Table 1). The distance from centre to centre of the opaque layers has been measured as $120 \pm 3 \text{ \AA}$ (SD = 14, Table 1). From these values the width of the separate opaque components and the distance between them were calculated to 40 and 80 $\text{\AA}$ respectively.

On the basis of the number of membranes in each cell and their extent in the cell, it is possible to arrive at a rough estimate of the area the membranes represent. If it is reckoned that each membrane has an extent of $2 \times 2 \mu$, then the area of that part of the membrane which is seen to be in contact with the layer between the basement membrane and the cell membrane will be about $10 \mu^2$. If, further, we reckon that the number of membranes in each cell is 34, the total area of the membranes will be about $340 \mu^2$. The free area of the cells is about $50 \mu^2$. Thus, the presence of the β -cytomembranes increases the surface of the cell about 7 times.

α -cytomembranes

The α -cytomembranes are defined (Sjöstrand 1956 c) as "about 50 $\text{\AA}$ thick membranes to one side of which opaque angularly shaped particles with a mean diameter of about 150 $\text{\AA}$ are attached. These membranes bound irregularly formed and, in most cases, very flat spaces".

The α -cytomembranes are encountered in the non-pigmented ciliary epithelium in groups of 10 to 20, mostly localised in one corner of the cell (Figs. 2 and 8). In most cases these groups are situated in the upper part of the cell, in very close relation to the cell membrane. In exceptional cases an assembly of membranes is found in the basal parts of the cell. Separate membranes may, however, be lying anywhere in the cytoplasm. The membranes are mostly orientated along the longitudinal axis of the cell.



Fig. 1. Survey picture of non-pigmented cells with parts of the nuclei (n). Between the cells the posterior chamber (pc) makes an indentation. Around this several β -cytomembranes (β -cm) are arranged. The mitochondria (m) are diffusely spread in the cytoplasm. In the lower right corner the cells border (cb) on the pigment epithelium. Magnification 25,000 x.

Inset: Light micrograph of a ciliary process with posterior chamber (1), non-pigmented epithelium (2), pigment epithelium (3) and blood vessels (4). Magnification 1,300 x.

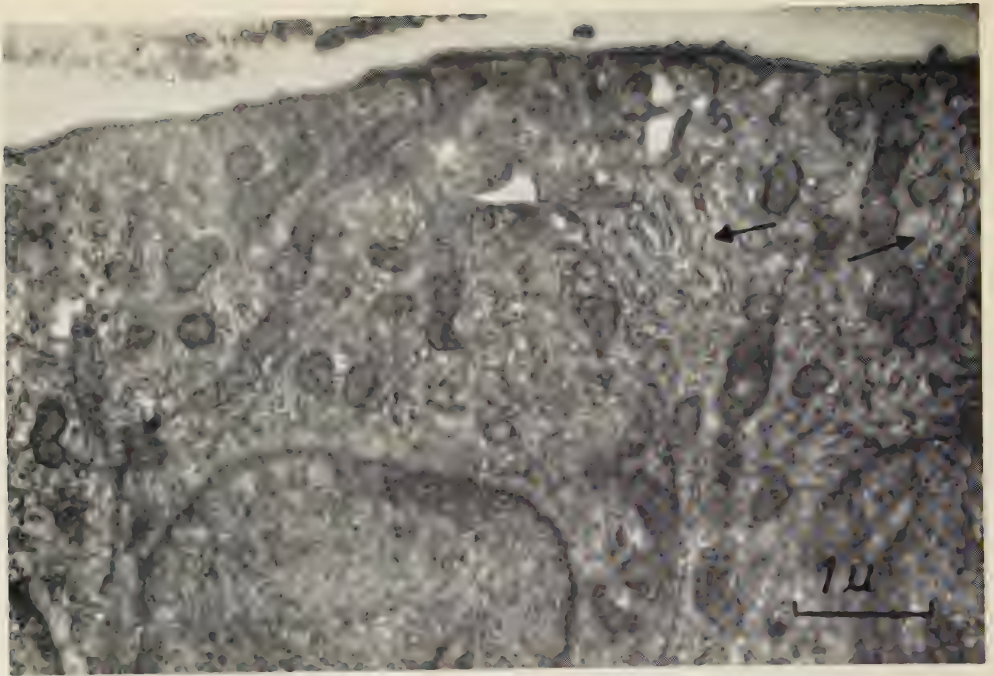


Fig. 2. (The upper picture). In this section no β -cytomembranes are seen. Several α -cytomembranes (arrows) lie in groups. Magnification 17,000 x.

Fig. 3. (The lower picture). In this section several β -cytomembranes (arrows) are seen in the cytoplasm. Magnification 17,000 x.

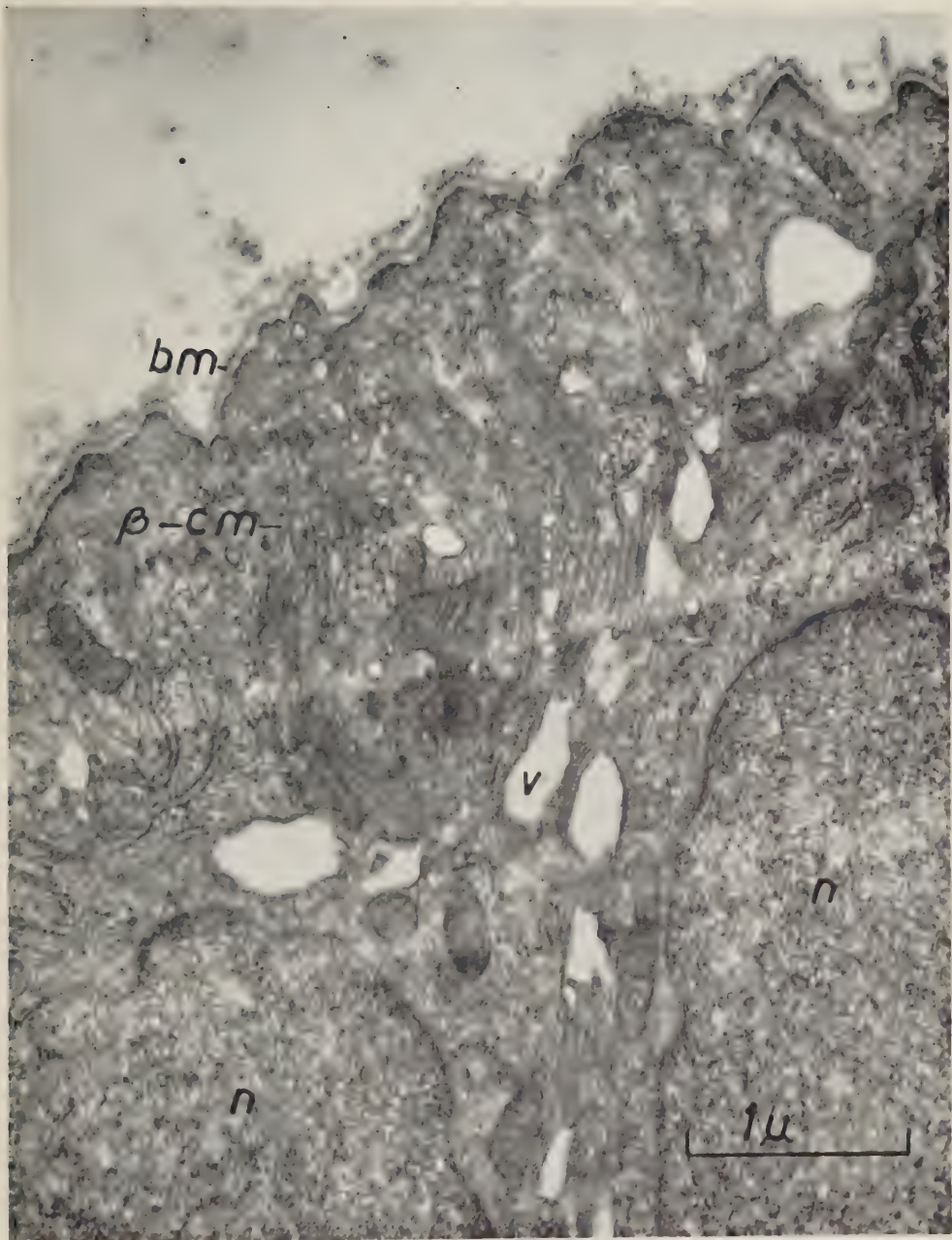


Fig. 4. Survey of the apical part of two non-pigmented cells with their nuclei (*n*) and with a basement membrane (*bm*) outside the free surface of the cells. Some β -cytomembranes (β -cm) and large vacuoles (*v*) lie in the cytoplasm. Magnification 30,000 $\times$.



Fig. 5. Some β -cytomembranes around an indentation of the posterior chamber (pc). The β -cytomembranes represent folds of the cell membrane.
Magnification 85,000 x.

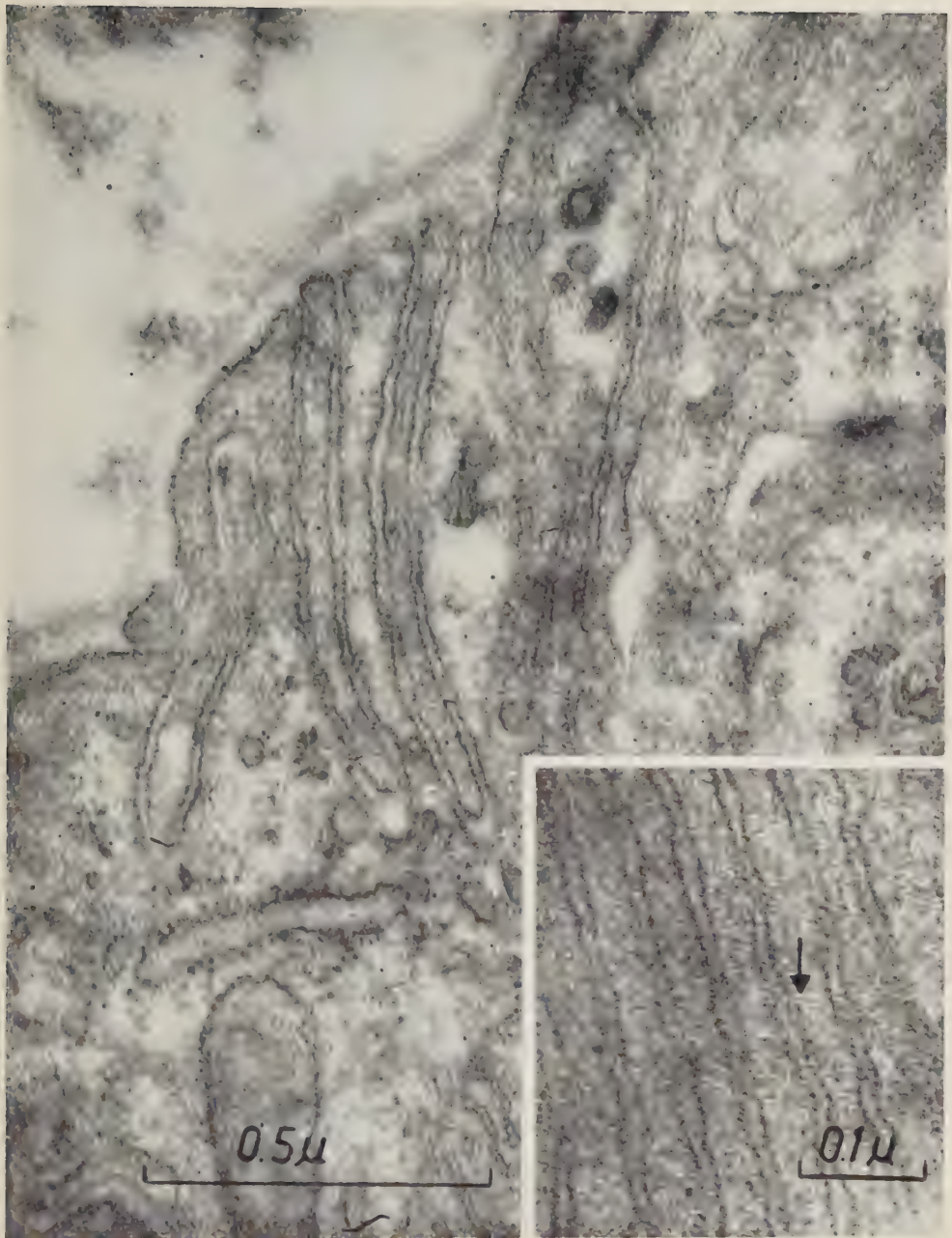


Fig. 6. Some β -cytomembranes showing their relationship to the cell membrane. To the left an isolated loop is seen without contact with the cell membrane. Magnification 95,000 $\times$.

Inset: The arrow indicates a place where the opaque layer in the β -cytomembrane appears to consist of three layers. Magnification 167,000 $\times$.

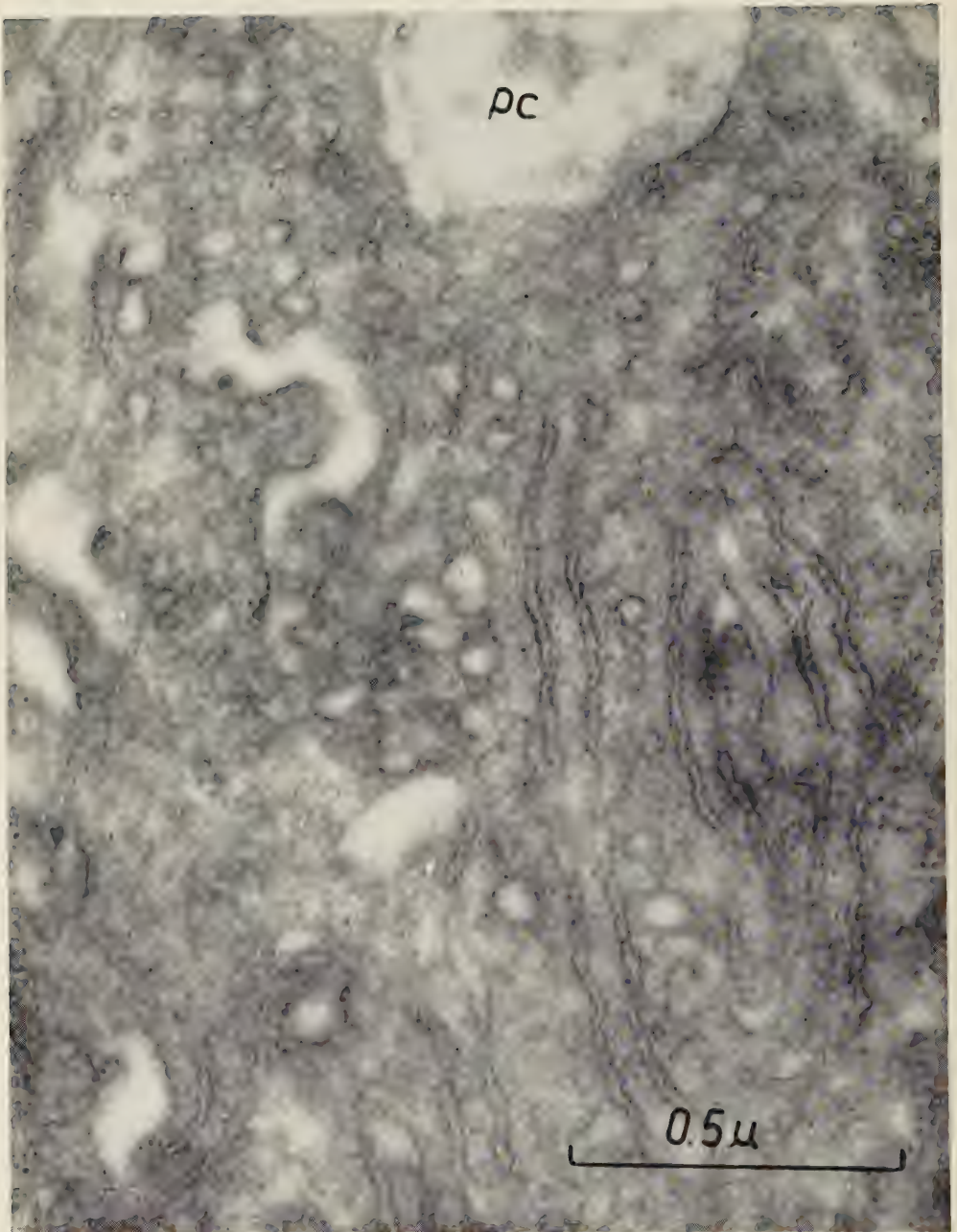


Fig. 7. β -cytomembranes not in contact with the cell membrane or the posterior chamber (pc). In the cytoplasm several vesicles lie in rows parallel with the β -cytomembranes. Magnification 90,000 $\times$.



Fig. 8. α -cytomembranes located in a limited area of the cell. There are no communications between the membrane pairs.
Magnification 56,000 x.

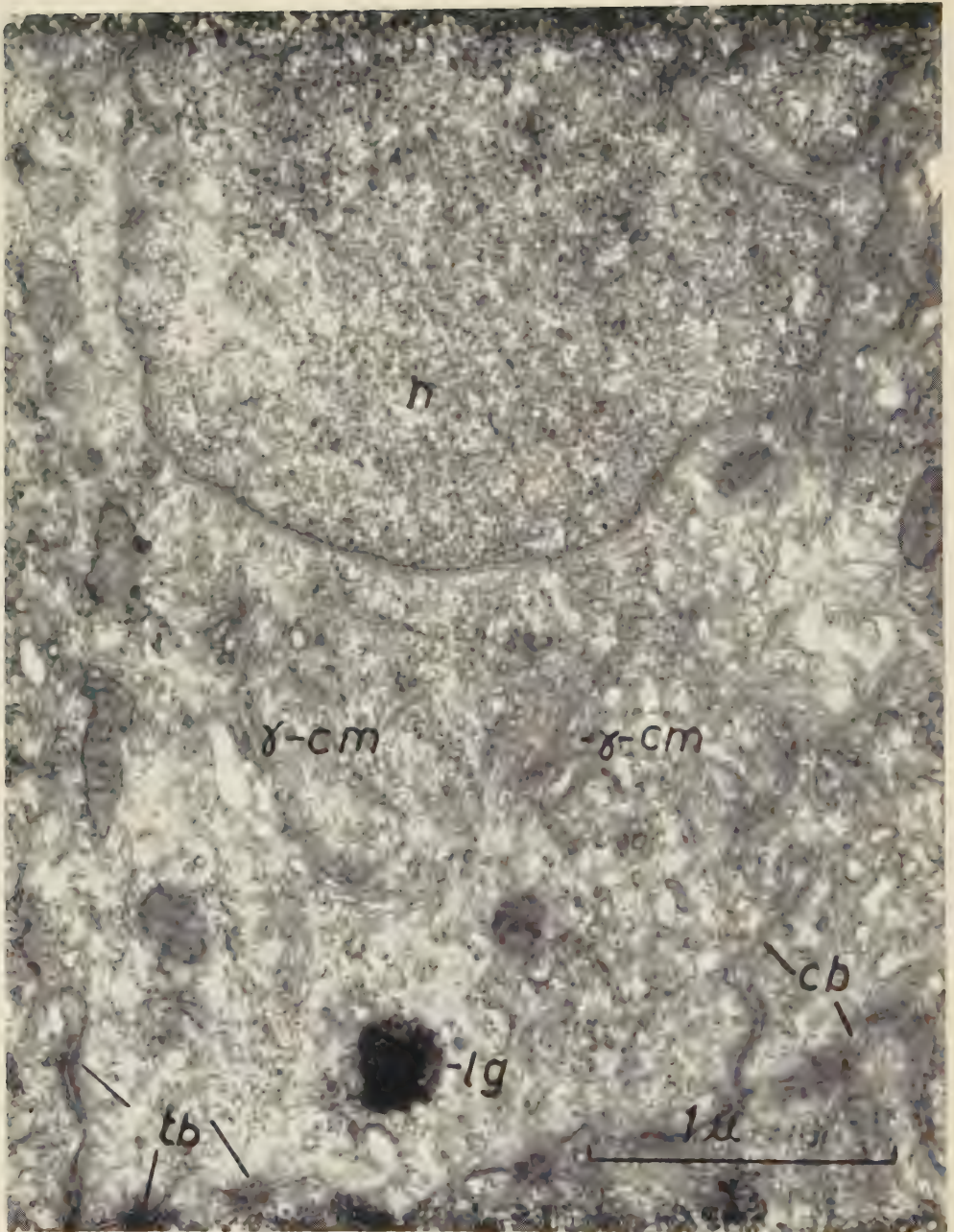


Fig. 9. Survey of the basal part of a non-pigmented cell. Below the nucleus (n) two Golgi units (γ -cm) are seen. On the cell border (cb) several terminal bars (tb) are seen. In the cytoplasm are large, strongly osmiophilic granules (lg). Magnification 42,000 $\times$.

Cell components	Number of animals	Number of cells	Number of components	The total thickness		The distance between the centers of the two osmophilic layers		The thickness of the osmophilic layer	The thickness of the osmophobic layer
				Mean	SD	Mean	SD		
β -cytomembrane	9	19	30	$160 \pm 3 \text{ \AA}$	15	$120 \pm 3 \text{ \AA}$	14	$^{140} \text{ \AA}$	$^{180} \text{ \AA}$
α -cytomembrane	5	6	19					$^{240} \pm 1 \text{ \AA}$	$^{180-760} \text{ \AA}$
γ -cytomembrane	6	9	9	$160 \pm 4 \text{ \AA}$	11	$110 \pm 3 \text{ \AA}$	9	$^{150} \text{ \AA}$	$^{160} \text{ \AA}$
Mitochondrion	10	32	465	$0,18 \pm 0,005 \mu$	0,026				
inner membrane	8	19	19	$150 \pm 4 \text{ \AA}$	17	$110 \pm 3 \text{ \AA}$	14	$^{140} \text{ \AA}$	$^{170} \text{ \AA}$
outer membrane	8	17	19	$130 \pm 4 \text{ \AA}$	15	$90 \pm 3 \text{ \AA}$	13	$^{140} \text{ \AA}$	$^{150} \text{ \AA}$
Nuclear membrane	8	11	11	$190 \pm 7 \text{ \AA}$	24			inner comp. $^{270} \pm 2 \text{ \AA}$ outer comp. $^{245} \pm 4 \text{ \AA}$ $40 \pm 1 \text{ \AA}$	$^{275} \pm 5 \text{ \AA}$
Cell membrane	8	13	13						
Intercellular border	5	5	5	$160 \pm 3 \text{ \AA}$	6	$120 \pm 2 \text{ \AA}$	5	$^{140} \text{ \AA}$	$^{180} \text{ \AA}$
Nuclear particles	7	13	10/nucleus	$150 \pm 3 \text{ \AA}$	12				
Free cytoplasmic particles	9	13	10/cell	$150 \pm 8 \text{ \AA}$	29				
Particles attached to the α -cytomembrane	5	6	15/cell	$150 \pm 3 \text{ \AA}$	8				

¹ Calculated ² Measured

Table 1: Quantitative data of some of the cell components.

The membranes are made up of closed units consisting of membranes arranged in pairs, provided with opaque particles on the outside. Each membrane pair encloses an area of varying magnitude, apparently without structure and with a density which is approximately the same as that of the surrounding cytoplasm. No communication between adjacent membrane pairs could be demonstrated in any one case.

The length the membranes extend in the cell varies very considerably. The longest membrane encountered has a length of $1.75\ \mu$ whereas the smallest only measures $0.18\ \mu$. The distance between the separate components of a membrane pair is likewise extremely varying which means that the space enclosed by the membranes has an irregular shape. The width of this varies between 180 and 760 Å. The separate components of the membranes, however, are extremely uniform. Measurements carried out on 19 membranes in 6 cells altogether from 5 animals give a value for their width of $40 \pm 1\ \text{Å}$ (SD = 1, Table 1).

The particles which are attached to each membrane have a rounded or triangular form. They are very irregularly distributed on the membranes. On long stretches they are entirely lacking. The magnitude of these particles is $150 \pm 3\ \text{Å}$ (SD = 8, Table 1), obtained in measurements made on 6 cells from 5 animals. They seem to be homogeneous and appear to be of the same type as those to be found plentifully scattered freely in the ground substance of the cytoplasm.

The Golgi apparatus

No description of the position of the Golgi apparatus in the ciliary epithelium or its appearance could be found in the literature. There has, however, been observed in this investigation a structure, which, judging from its appearance on the sections, agrees very well with what has been designated as the Golgi apparatus in other cells investigated.

This structure is encountered exclusively in the basal parts of the non-pigmented cells (Fig. 9), mostly located between the nucleus and the basal cell membrane, sometimes between the distal third of the nucleus and the intercellular boundary. Thus, it lies in very close relation to the nucleus (Fig. 10). In no case was it encountered apically to the nucleus. It occurs in a small restricted region, mostly as one unit, sometimes as two separate units, and more seldom as three or more units.

Each unit is made up of 2 or possibly 3 components: a membrane system, the membranes named γ -cytomembranes (Sjöstrand 1956 c), a number of

vesicle-like formations, in this investigation named vesicles, and possibly a special ground substance.

The membrane system is built up of a number of membranes arranged in pairs. Each pair forms the border of a closed area, i. e., the membranes of a pair fuse along their rims. The membranes lie in 3—6 rows running parallel, with 4—8 pairs in each row (Fig. 10). Connections at the rims between pairs lying one after the other have been observed but no communication seems to exist between pairs lying parallel to each other.

The closed region bounded by a membrane pair is of varying shape. Mostly it has the character of a long and narrow space between 150 and 350 Å wide and between 700 and 2,500 Å long, displaying a diffuse structure without any particular organisation. New and then, however, there are widened spaces with the character of large vacuoles, measuring up to 0.2 μ in diameter. No osmiophilic substance was observed inside these vacuoles.

If the distance between the separate components of a membrane pair thus varies quite considerably, the distance between adjoining pairs is exceptionally regular. It measures about 60 Å while the separate components in a membrane pair measure about 50 Å (Table 1).

In immediate connection with the membrane system described above there are observed assemblies of isolated vesicles (Fig. 10), round or oval in shape, with a density in the inner structure increasing from the centre to the periphery and separated from the surroundings by a single membrane about 50 Å wide. The size of the vesicles varies from 250 to 700—800 Å. In size, shape, and build they display great resemblance to the vesicles found diffusely scattered in the cytoplasm in other parts of the cell.

Whether there exists any special ground substance in the Golgi apparatus cannot be surely decided. The fixation method employed in the present investigation gives generally a very opaque ground substance over the whole cell so that it is difficult to decide whether the ground substance occurring in the Golgi region differs from the ground substance in other parts of the cell. When the fixation is not so good, i. e., where the ground substance of the cytoplasm is less dense due to swelling, the ground substance of the Golgi apparatus is more opaque than that of the rest of the cytoplasm.

The cell membrane and the intercellular border

The free surface of the ciliary epithelium, i. e., that part which borders on the posterior chamber, is bounded by a single membrane. Its relation to the β -cytomembranes is described in the chapter concerning these and will not be dealt with here.

In those parts of the free cell surface where β -cytomembranes do not occur, the width of the opaque layer which bounds the cell and which represents at least one layer of the cell membrane has been measured as $40 \pm 1 \text{ \AA}$ for 13 cells from 8 animals ($SD = 2$, Table 1).

In the boundary layer between adjoining cells the respective cell membranes are separated by a less opaque space. The total width of this double membrane has been measured for 5 cell boundaries from 5 animals as $160 \pm 3 \text{ \AA}$ ($SD = 6$, Table 1). The distance between the centres of the opaque layers has been measured as $120 \pm 2 \text{ \AA}$ ($SD = 5$, Table 1). From this the width of the opaque layers has been calculated to $40 \text{ \AA}$ each, and the distance between them as $80 \text{ \AA}$.

This last-named layer is as a rule very uniform but at isolated spots it enlarges to vacuole-like formations which most usually occur in the apical parts of the boundaries. No definable structure in this layer has been observed, and the vacuolar enlargements appear to be empty.

The boundary between the cells displays many folds and irregularities. The folds may either be directed at right angles to the boundary surface or, which is more usual, obliquely or almost parallel to it. This arrangement of the intercellular boundaries is most common in the upper two thirds of the cells whereas in the basal parts they run more straight (Fig. 11). At those spots where the intercellular boundaries have the arrangement described above, and where therefore double membranes in varying number lie close to each other, it can be observed that in the space between the membranes there is an additional opaque layer. It is single and it shows a lower contrast than the opaque components of the double membranes (Fig. 12). Moreover, it appears to be thinner and more diffuse than them.

Terminal bars

In the basal portion of the intercellular border there are regularly encountered 2—3 regions, in which the cytoplasm situated nearest the cell membrane displays a strong osmiophilia. They represent the so-called "terminal bars".

The boundary line between the non-pigmented and the pigmented cell layer (Figs. 1 and 13) is in principle built up in the same manner as the boundary between the non-pigmented cells. The main components are the cell membranes of the different cells, which together form a double contoured membrane with a central lighter layer. It has two features which distinguish it from the cell boundaries referred to above. It displays an appreciably more

even course with only a few small fold formations. Most characteristic, however, is the plentiful occurrence of terminal bars. These occur very regularly to a number of 5—7 in a stretch corresponding to the diameter of a cell belonging to the non-pigmented layer.

As stated, a terminal bar is characterised by a region connected with a cell boundary where the osmiophilic tendency of the cytoplasm is much greater than elsewhere. In the present case, this region is between 0.12 and 0.26 μ long and between 300 and 700 Å wide. The distance between each terminal bar varies between 0.4 and 0.5 μ . The osmiophilic layer of the cell membrane pass unbroken through each terminal bar region. They stand out less distinctly in this portion, however, owing to poorer contrast conditions. The distance between the opaque layers is greater in a terminal bar than in other portions of the same cell boundary. Measurements have been made on 21 terminal bars from 13 cells and 6 animals. The total thickness of the cell boundary is 220 ± 5 Å (SD = 21). The distance between the centres of each membrane is 180 ± 5 Å (SD = 22). Thus each membrane is 40 Å and the distance between them 140 Å. Equivalent measurements of the cell boundary between terminal bars give the following values for the same number of cells and animals: total thickness 140 ± 4 Å (SD = 11), the distance from the centres of the membranes 100 ± 3 Å (SD = 11), each membrane is 40 Å and the distance between them 60 Å. The difference in total thickness between these parts of the same cell border is 80 ± 6 Å. There is, therefore, a significant difference which is associated with an increase in width of the central lighter layer.

In high resolution material it was observed that the opaque component of the cell membrane within a terminal bar is made up of three layers, two opaque ones surrounding a light central layer, all of them about 20 Å wide (Fig. 14). The observations were few in number and the more detailed relationships between these components was not closely analysed.

The basement membrane

A structure resembling in size and appearance what is generally called basement membrane was encountered in connection with the cells in the ciliary epithelium. It consists of a continuous membrane covering the free surface of the cell. It is rather diffuse and displays a low contrast. The thickness varies from 290 to 370 Å with a mean of 310 ± 6 Å (SD = 22).

Between this membrane and the cell membrane there is observed a light layer varying between 400 and 700 Å in width. In this layer there may be

observed transverse opaque bands, which appear to bind the two membranes to each other (Fig. 15). In respect of structure and contrast conditions these bands agree with the basement membrane, in which no definite structure could be detected.

Mitochondria

In the non-pigmented part of the ciliary epithelium, the mitochondria consist of comparatively small, cylindrical bodies distributed in a diffuse manner in the cell. As may be expected, in view of the different directions of sectioning, the length shows very considerable variation. The longest mitochondrion measured was 2.3μ . It was possible, however, accurately to determine the width of these mitochondria. It is $0.18 \pm 0.005 \mu$ (SD = 0.026, Table 1), obtained in the measurement of 465 mitochondria in 32 cells from 10 animals. About 15 mitochondria were measured in each cell, and the mean of these was taken for computing the mean for them all.

No definite orientation of the mitochondria in the cell could be observed.

The mitochondria are surrounded by an outer membrane made up of three layers, two opaque ones separated by a light middle one (Fig. 18). The total thickness of the outer membrane is $130 \pm 4 \text{ \AA}$ (SD = 15, Table 1). The distance between the centres of the opaque layers is $90 \pm 3 \text{ \AA}$ (SD = 13, Table 1). From this the width of each opaque layer was calculated as $40 \text{ \AA}$ and that of the central layer $50 \text{ \AA}$.

The inner structure consists of two components, viz., a varying number of membranes arranged in parallel and a homogeneous ground substance. The inner membranes are as a rule oriented in a plane at right angles to the outer membranes, and like them consist of three layers, two opaque and a middle light layer. The total thickness of the inner membranes is $150 \pm 4 \text{ \AA}$ (SD = 17, Table 1). The distance from the centres of the opaque layers is $110 \pm 3 \text{ \AA}$ (SD = 14, Table 1). From these values there are obtained the width of each opaque layer as $40 \text{ \AA}$ and the width of the central layer as $70 \text{ \AA}$. In comparing the thicknesses of the outer and inner membranes it appears as if the latter are somewhat thicker. The difference is small, however, and not significant.

The extension of the inner membranes in the ground substance varies considerably. In exceptional cases, membranes may be seen lying free without contact with the outer membrane. The rule is, however, that one or both ends are in contact with that membrane. A calculation indicates that 40 % extend over the whole width of the mitochondrion or more than 3/4 thereof, 30 %

between $1/2$ and $3/4$, 18 % between $1/4$ and $1/2$, and 12 % less than $1/4$ of the width of the mitochondrion.

As a rule there does not appear to exist any communication between the middle layers of outer and inner membranes but such has been observed in a few cases. Usually this arrangement occurs only at one end of the inner membrane but in a single case it was observed that both ends are joined in this way with the outer membrane.

What is stated above regarding the appearance of the mitochondria applies to most of them. Exceptions were observed, however. For instance, y-shaped mitochondria were observed as also mitochondria with small processes at the sides. These variations apply to the shape. Another departure from the usual structure is concerned with the arrangement of the inner membranes. In some cases these have quite a different arrangement than is usually the case. Instead of a number of inner membranes set at right angles to the outer membrane, there may be seen one (Fig. 20), two (Fig. 19), or several (Fig. 21) located centrally in the mitochondrion and parallel to the outer membrane. They seem to extend from pole to pole over the whole length of the mitochondrion. Each of these membranes appears to be closed at the ends without contact with the outer membrane. Besides these membranes there may be observed in the same mitochondrion other membranes arranged in the usual way. These, however, are very short. They do not reach the central parts of the mitochondrion and do not appear to have contact with the membrane or membranes situated in the centre. This type of mitochondria, moreover, differs from the ordinary type by the inner membranes being appreciably thicker than the outer membranes, both as regards total thickness, amounting to around 200 Å, and the thickness of the opaque components, amounting to around 60 Å. The dimensions of the outer membrane, on the other hand, are the same when compared with other mitochondria.

Finally, there were observed some mitochondria where the inner membranes in one part of the mitochondrion are arranged in the manner stated above, whereas in another part they have this extent only in about half the length of the mitochondrion. In other parts of the same mitochondrion there are encountered membranes arranged in the ordinary manner, at right angles to the longitudinal axis of the mitochondrion (Figs. 22 and 23).

The ground substance of the mitochondria has a homogeneous appearance with an even distribution. No structure was seen.

Most of the mitochondria in the ciliary epithelium lack the opaque regions which characterise mitochondria of some other cell types. Only in exceptional cases were there observed such strongly osmiophilic granules between the inner membranes of the magnitude of 250—300 Å.

The nucleus

The nucleus is situated in the basal parts of the cell. It has mostly an oval form but the nuclear membrane often displays one or more deep invaginations (Fig. 9). These may sometimes be so large that in some sections it is possible to see two nuclear parts entirely separated from each other.

The nucleus is enclosed entirely in a membrane. The nuclear membrane is made up of three components, two of which are strongly osmiophilic. Between them lies the third which is less osmiophilic. The inner component is wider than and displays a density that is appreciably greater than that of the outer one (Fig. 26). Both components are smooth. Measurements made on 11 cell nuclei from 8 animals gave an average width of the inner component as $70 \pm 2 \text{ \AA}$ (SD = 7, Table 1) and of the outer component as $45 \pm 4 \text{ \AA}$ (SD = 15, Table 1). The difference in width is significant. No granulation in the inner component, which could account for the observed difference in thickness, was present. The distance between the two components was measured on the same nuclei listed above, and the average obtained was $75 \pm 5 \text{ \AA}$ (SD = 17, Table 1). The total width of the nuclear membrane measured separately, averaged $190 \pm 7 \text{ \AA}$ (SD = 24, Table 1). The distance between the outer and inner components was mostly very regular. Now and then, however, there were seen suggestions of small bulges but any large ones which may be designated as fold formations were not observed.

At restricted areas very few in number, there may be observed "holes" in the nuclear membrane in regions 250—500 Å in size (Fig. 26). At the edges of these "holes" the two opaque components fuse through a U-shaped connection. The structure in the regions where the nuclear membrane appears to be lacking is homogenous, without granules. Some observations indicate that the "holes" in the nuclear membrane is bridged over by a thin single membrane (Fig. 26). In thickness it measures 30—35 Å and displays a contrast which is appreciably weaker than that of the nuclear membrane in general. It was not possible to discover in this investigation any ring formations such as have been observed on tangential sections by several investigators.

As regards the nuclear membrane, it may be stated in conclusion that nowhere were there observed either direct communications or other structures suggesting such a communication with the other cell components.

The nuclear substance consists for the most part of particles which are uniformly distributed over the whole nucleus, except for a slightly denser concentration next to the nuclear membrane. Here they are often seen arranged in rows, giving a false impression that there exists a third nuclear membrane.

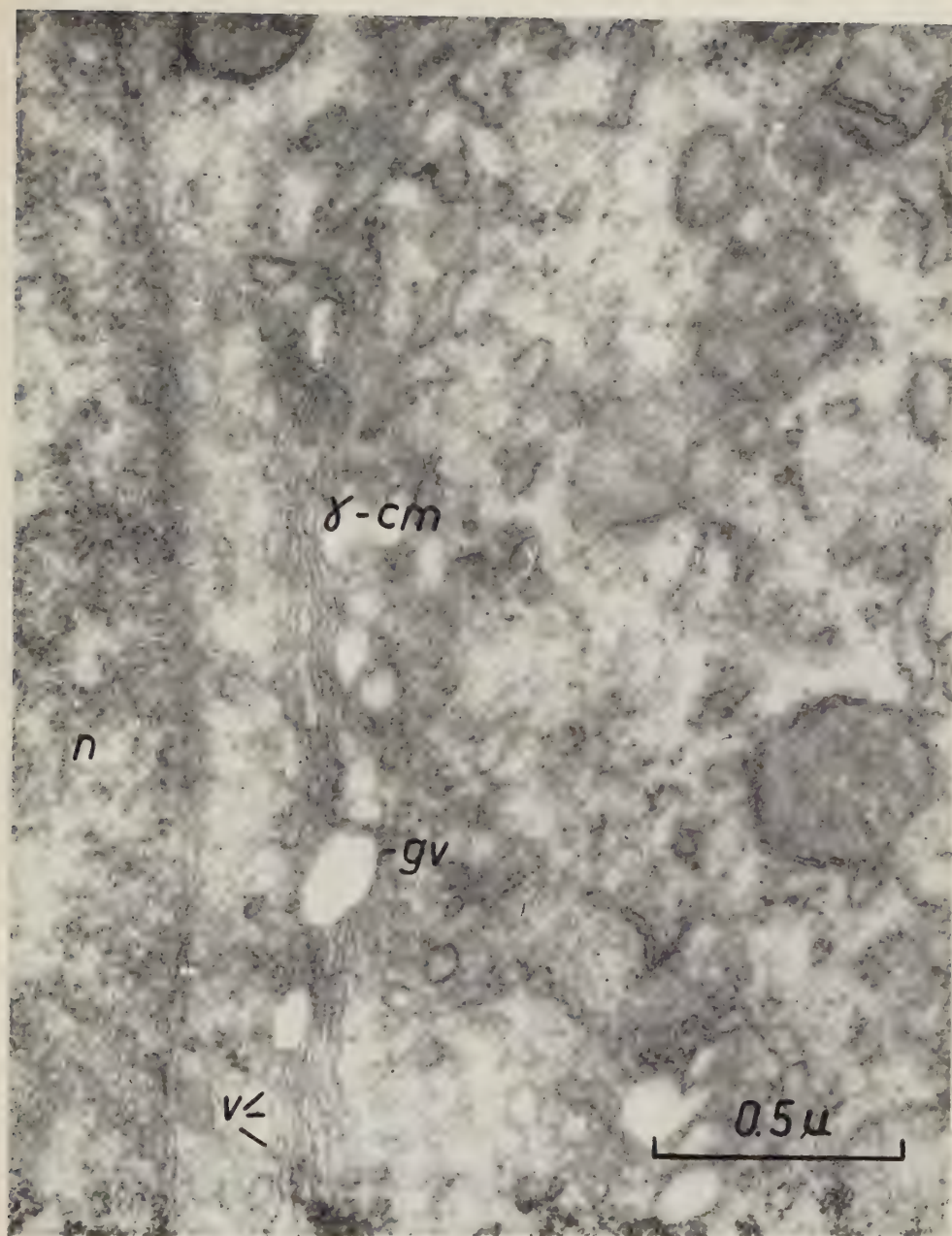


Fig. 10. Golgi apparatus. The membrane pairs (γ -cm) are arranged in rows. A few vacuoles (gv) and several vesicles (v) are seen. The Golgi apparatus lies close to the nucleus (n). Magnification 67,000 x.

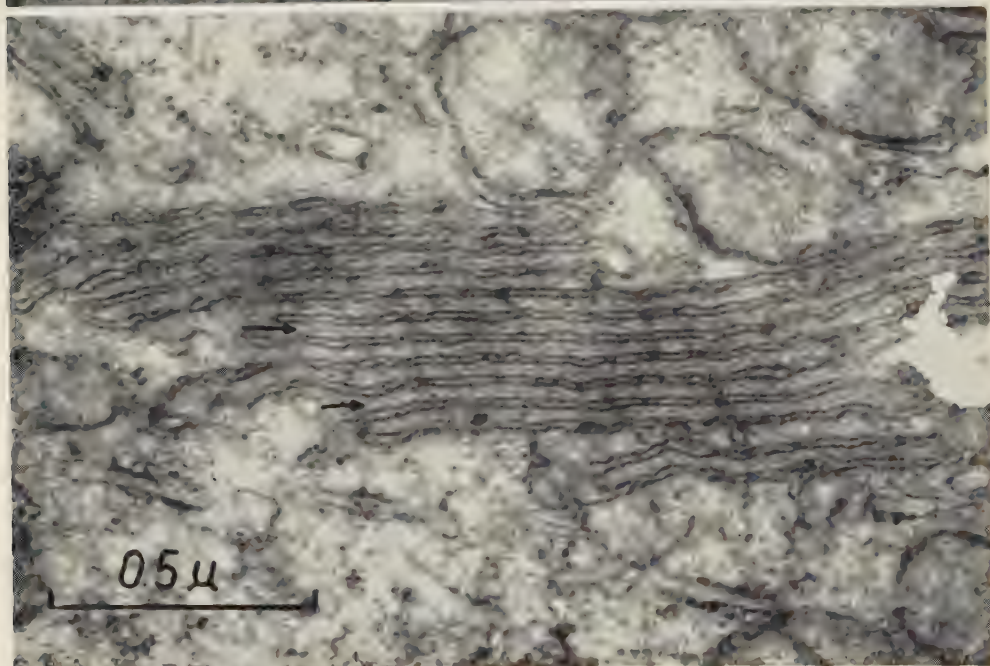
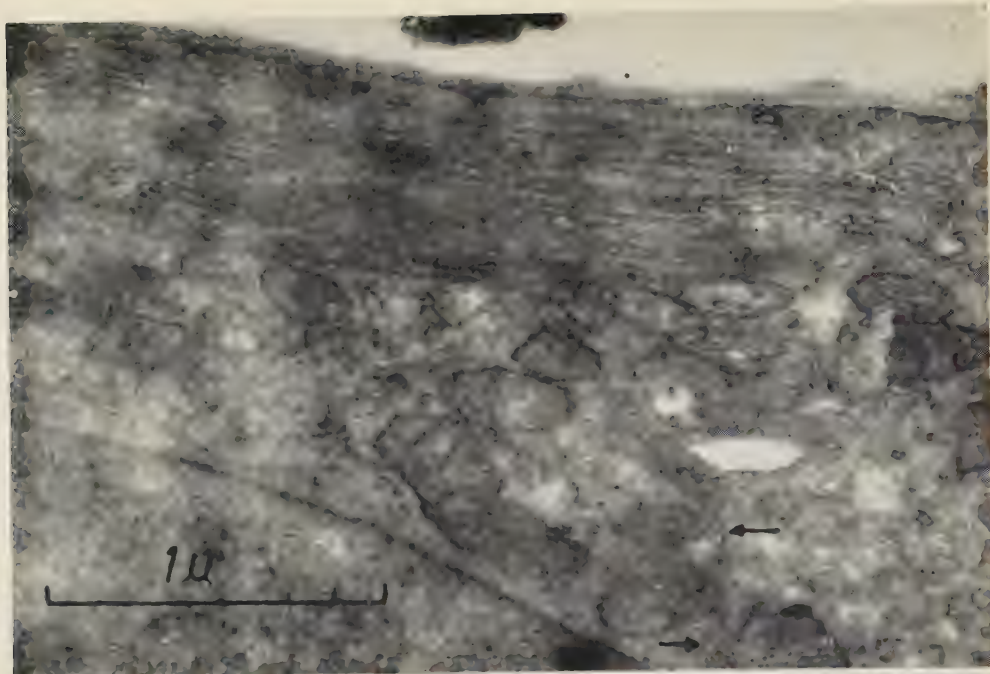


Fig. 11. (The upper picture.) Survey of a cell border showing its highly folded course in the apical part and its less folded course (arrows) towards the base of the cell. Magnification 36,000 $\times$.

Fig. 12. (The lower picture.) Higher magnification of a folded cell border. In the cytoplasm, extending into the folds, an opaque layer (arrows) is observed. Magnification 70,000 $\times$.

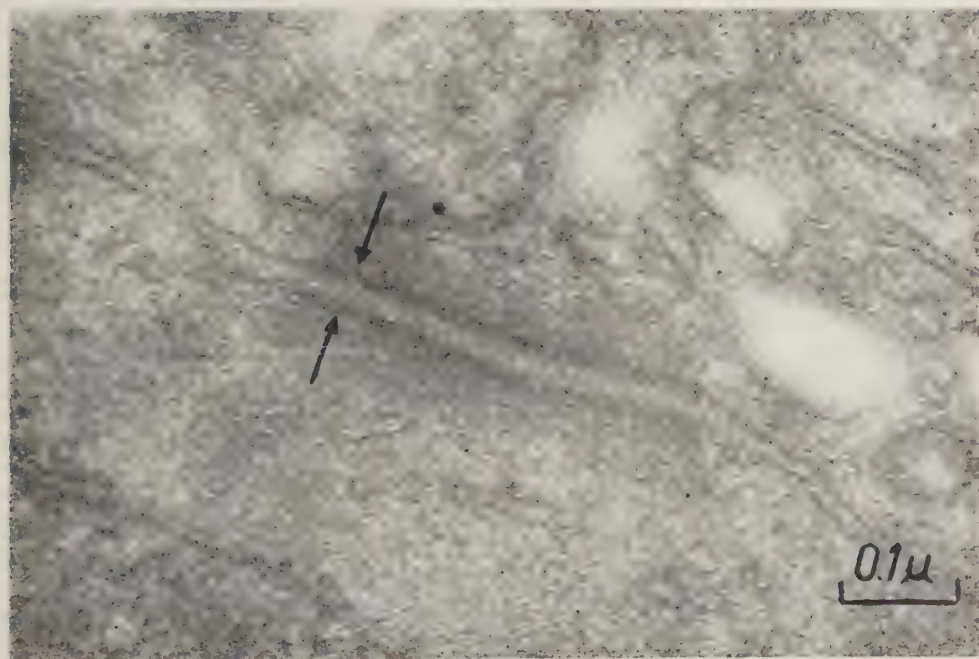
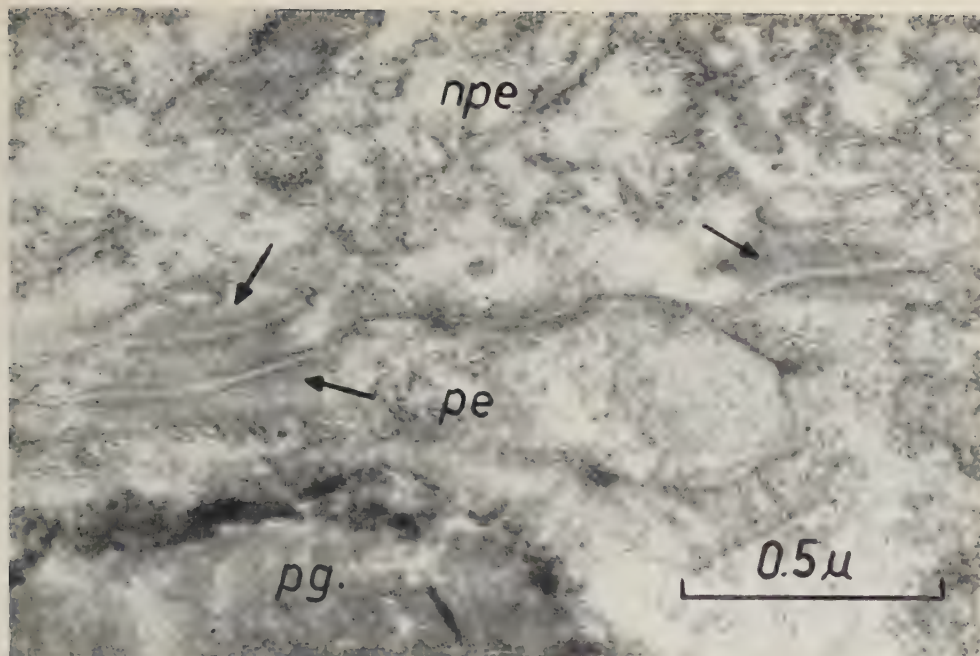


Fig. 13. (The upper picture.) Cell border between a non-pigmented cell (npe) and a pigmented cell (pe) with a pigment granule (pg). Some terminal bars are seen (arrows). Magnification 69,000 $\times$.

Fig. 14. (The lower picture.) A terminal bar at higher magnification. The opaque component of the cell membrane consists of three layers (arrows). Magnification 149,000 $\times$.

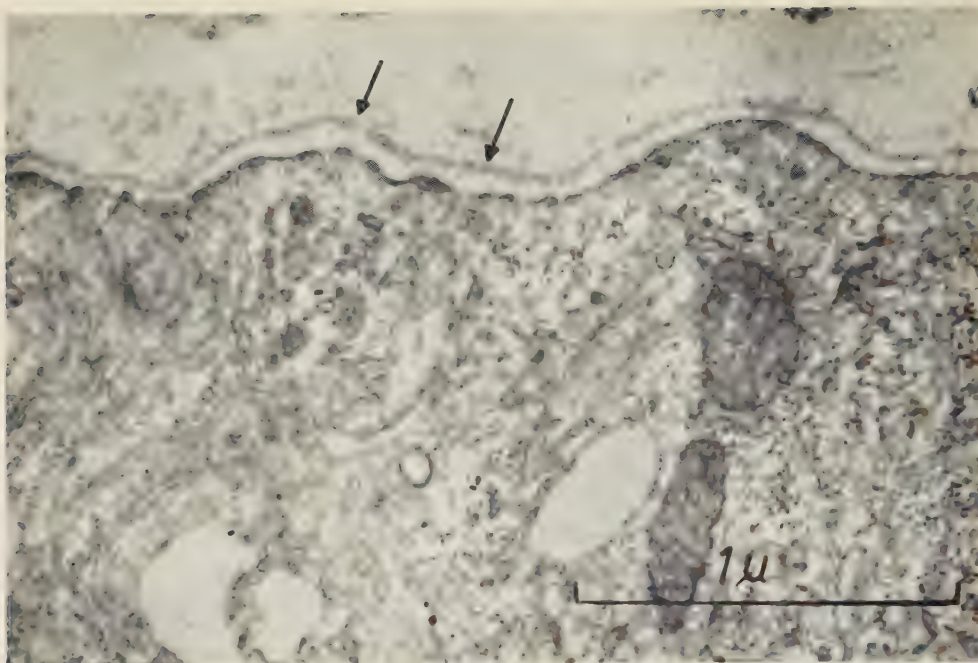


Fig. 15. (The upper picture.) The basement membrane (arrows) outside the free surface of the non-pigmented epithelium. Thin bands seem to connect the basement membrane with the cell membrane. Magnification 50,000 x.

Fig. 16. (The lower left picture.) Large spherical body, delimited by a double membrane. The ground substance of the body is less dense than the ground substance of the cytoplasm. Magnification 74,000 x.

Fig. 17. (The lower right picture.) Large spherical bodies with one solitary granule and some smaller particles in the ground substance. Magnification 77,000 x.

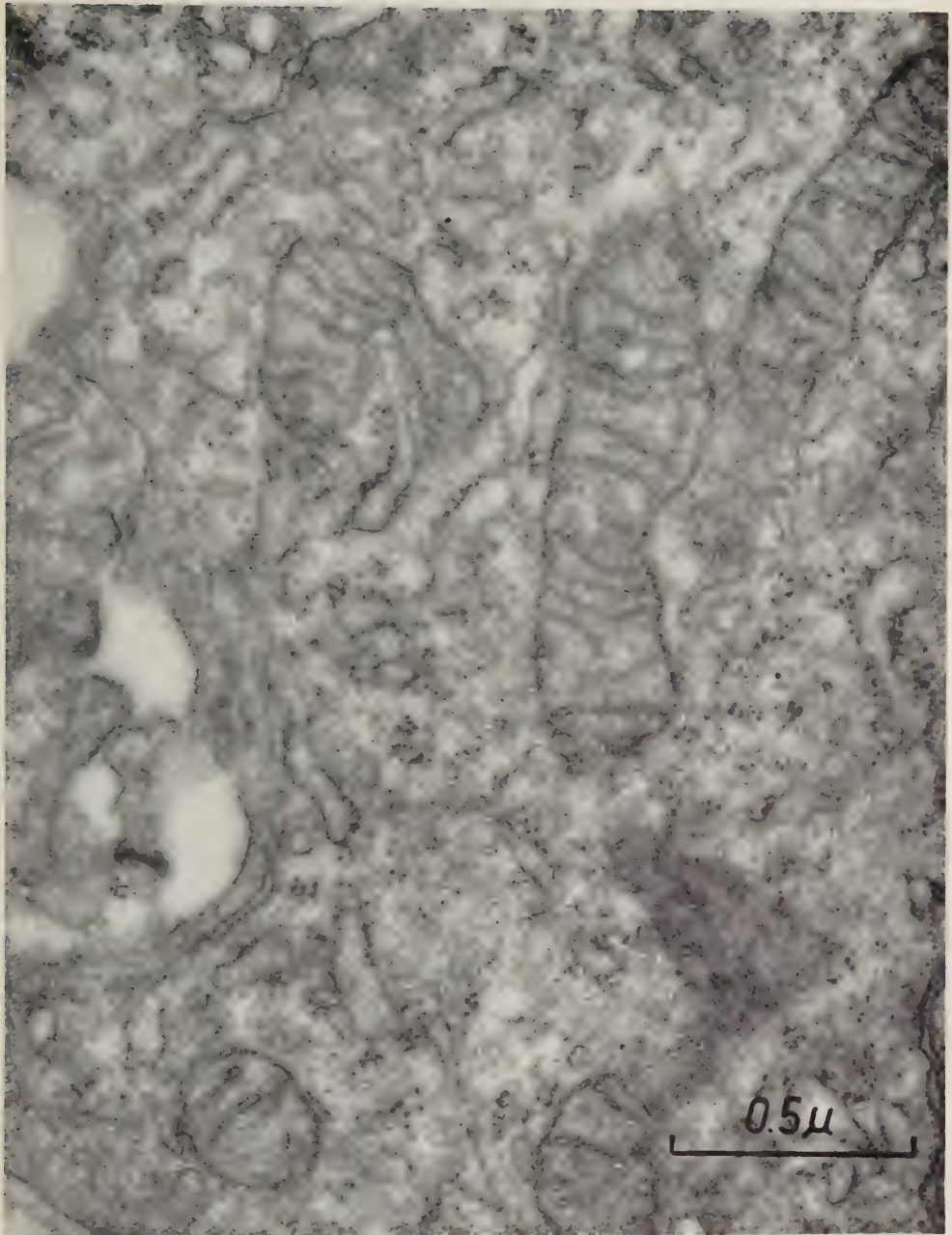


Fig. 18. Mitochondria from the non-pigmented epithelium. The inner membranes are arranged in the usual way, mainly at right angles to the long axis of the mitochondrion. Magnification 66,000 $\times$.

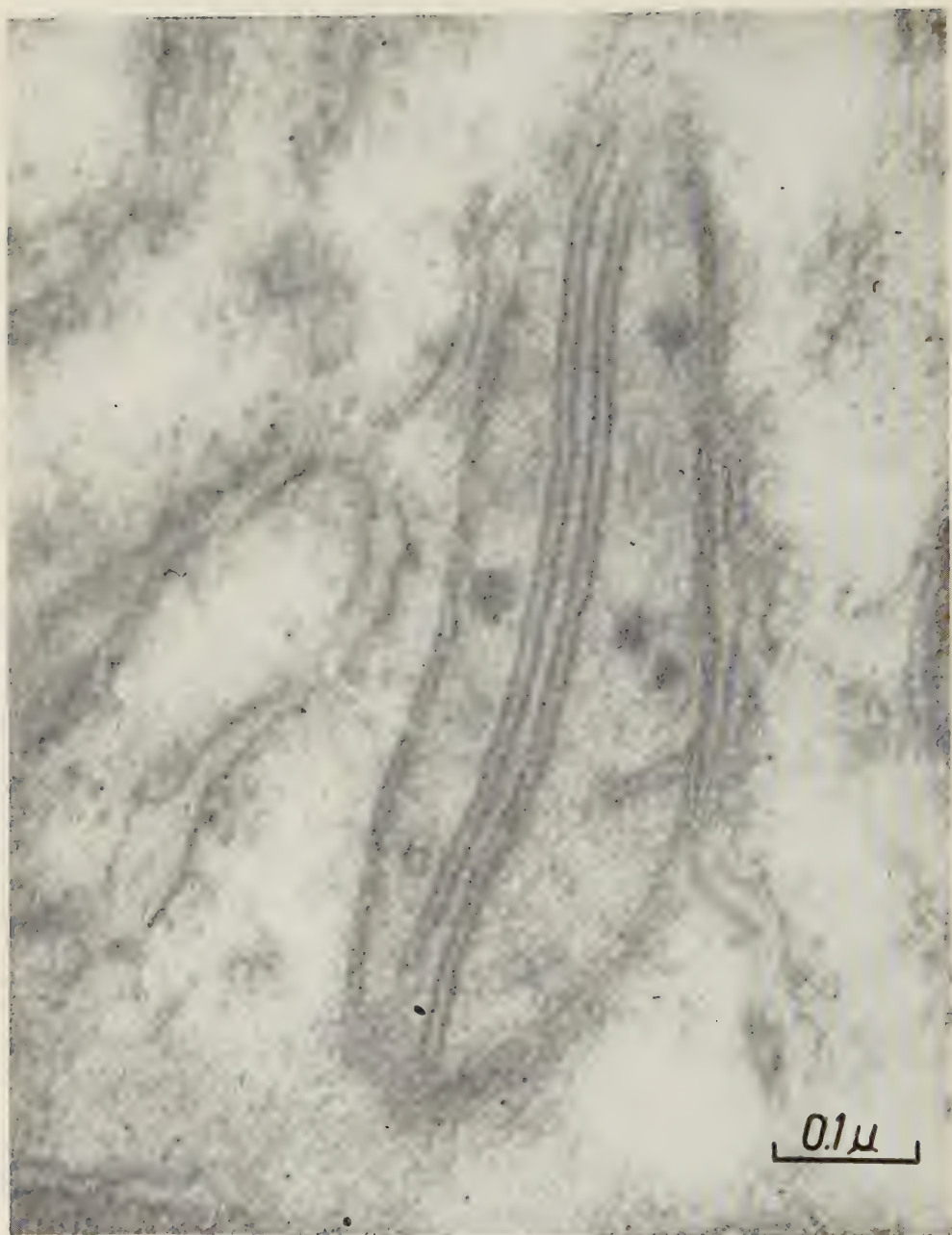


Fig. 19. Mitochondrion with two inner membranes parallel with the long axis of the mitochondrion.
Magnification 187,000 x.

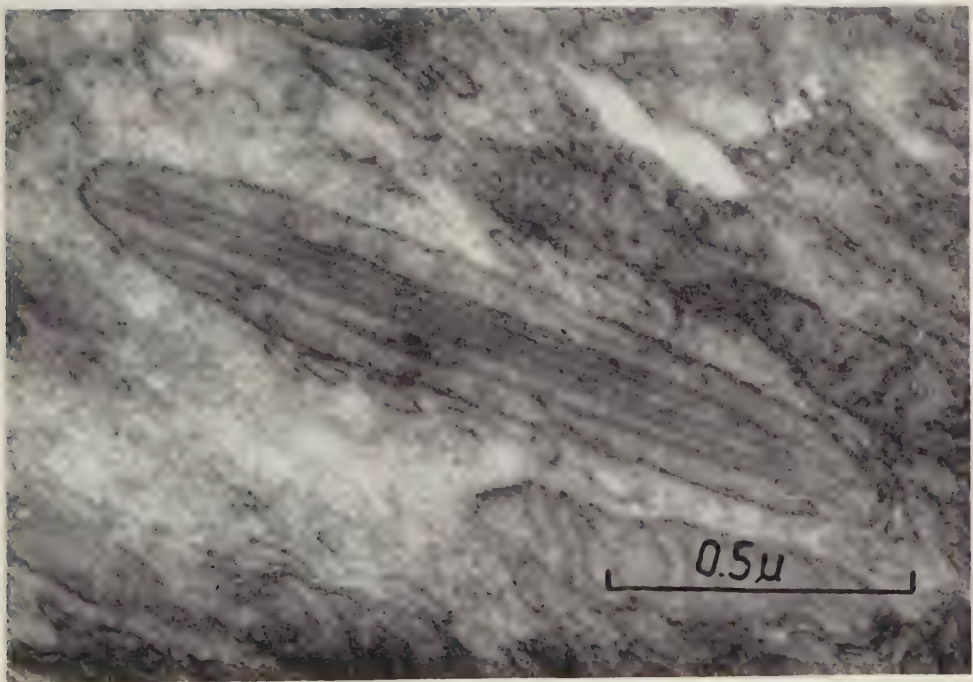
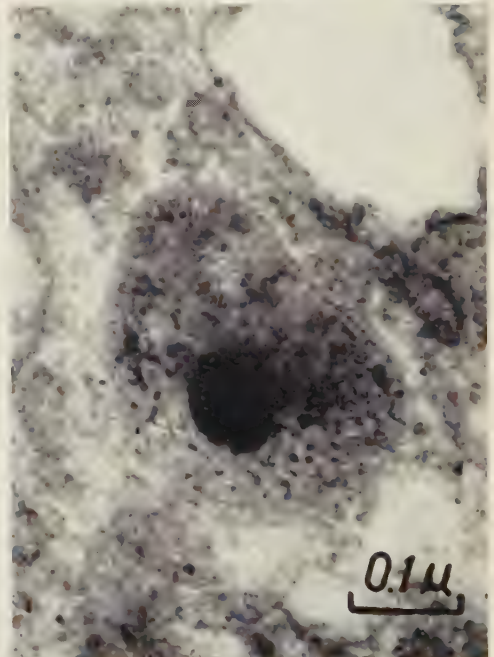
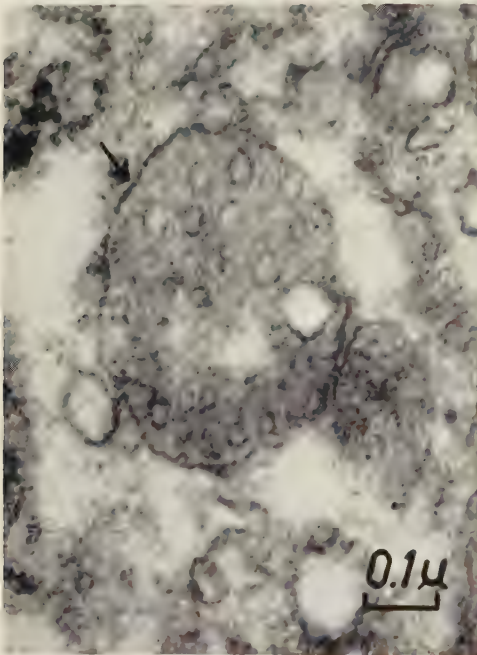
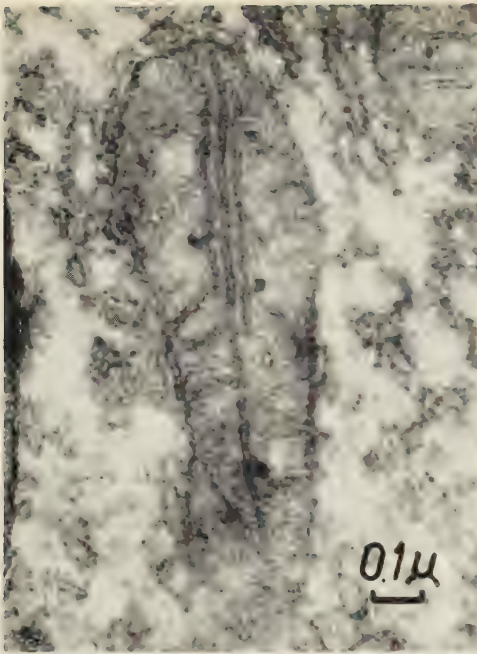


Fig. 20. (The upper picture.) Mitochondrion with one inner membrane arranged as in Fig. 19.
Magnification 110,000 x.

Fig. 21. (The lower picture.) Mitochondrion with several inner membranes arranged as in Fig. 19.
Magnification 77,000 x.



Figs. 22—23. (The upper pictures.) Serial sections through a mitochondrion showing different arrangement of the inner membranes in different parts of the mitochondrion. Magnification 60,000 x.

Fig. 24. (The lower left picture.) Large granule delimited by a double membrane (arrow). Several vesicles lie in the ground substance of the granule. Magnification 93,000 x.

Fig. 25. (The lower right picture.) Large granule with strongly osmiophilic grains in the ground substance. Enlarged detail of Fig. 35. Magnification 139,000 x.

Away from the membrane, the particles occur as a rule isolated from each other and distributed in diffuse manner. Here and there, however, there occur local concentrations of these particles. The particles are irregular, rarely round, most often they appear triangular or oval. No difference in density between the central and peripheral parts of each individual particles was observed. The mean size, obtained by measurement of 13 nuclei from 7 animals, was $150 \pm 3 \text{ \AA}$ (SD = 12, Table 1). 10 particles have been measured in each nucleus.

The nucleolus is to be found in the peripheral parts of the nucleus in the form of a darker well-defined region about $1.0 \times 0.7 \mu$ in size. It is often in close association with the inner component of the nuclear membrane. The boundaries of the nucleolus are very sharp but are without a distinct limiting membrane. The nucleolus is made up of tightly packed particles of the same size and shape as the other nuclear particles. They are packed together in wide communicating clusters, with lighter parts between, in which there are found isolated particles. The strong difference in contrast between the nucleolus and the remaining nuclear substance is entirely due to the particles in the former being very tightly packed. They do not seem to differ in osmiophilia.

Large granules

Two types of large granules occur in the ciliary epithelium. The first is represented by Fig. 24. Usually, only a single large granule of this type has been seen in a cell. In exceptional cases, two have been observed in the same section of a cell. It may be encountered anywhere in the cell, but always in close relation to the nucleus. The size varies between 0.11 and 0.29μ . In shape it is round or oval with a rather irregular boundary line, which consists of a triple-layered membrane, each layer about $20 \text{ \AA}$ thick. The inner structure varies from one granule to another. In some cases it seems to be filled with a homogeneous material without structure, in others there may be seen a number of round vacuole-like formations, $400\text{--}500 \text{ \AA}$ in size, provided with membranes. Some of these units display a central lighter zone.

The other type of granule appears multiple (Fig. 25). It was observed in practically all sections of the cells analysed, in some cases numbering 4—5 in the same section, so that it appears to be a cell component constantly occurring. It is mainly encountered in the basal parts of the cell between the nucleus and the basal cell boundary. The shape in the majority of cases is quite irregular. The sizes are extremely varying. The smallest diameter

measured was $0.14\ \mu$, the largest $0.35\ \mu$. The boundary consists of a membrane about $40\ \text{\AA}$. The most characteristic feature of this granule is its strongly osmiophilia so that it appears very pronounced in relation to the surrounding cytoplasm. The inner compact ground substance of these granules has a number of dark grains embedded in it, varying between 40 and $70\ \text{\AA}$ in size, sometimes assembled in large clumps or smaller aggregates, 250 — $300\ \text{\AA}$ in size.

Large spherical bodies

Besides the large granules, there was observed a relatively large rounded body represented in Figs. 16 and 17. It was observed in only a few cells so that it may be that it is not a constituent occurring in all cells. Otherwise, there was not observed any difference between the cells, in which it occurs, and the other non-pigmented cells. In the case where it was encountered, it was invariably localised very near the free surface of the cell. It is oval in shape, with its least diameter varying between 0.23 and $0.48\ \mu$ and the largest varying between 0.47 and $1.0\ \mu$. The boundary consists of a double membrane about $150\ \text{\AA}$ wide, with two outer opaque layers about $40\ \text{\AA}$ wide and a central light layer about $70\ \text{\AA}$. In the inside of the organelle a number of units are found, of which at least four types may be distinguished. There is a ground substance with a density considerably less than that of the surrounding cytoplasm. There is a single granule 900 — $1200\ \text{\AA}$ in size and bounded by a membrane. Further, there occurs a number of smaller particles 350 — $450\ \text{\AA}$ in size, and finally small particles $150\ \text{\AA}$ in size, of the same appearance as those occurring freely in the ground substance of the cytoplasm.

The ground substance of the cytoplasm

The space in the cell between the cell components described above is filled with a substance, in which 3 different constituents may be distinguished. There is a diffuse scattering of small homogeneous angular particles. They occur in all parts of the cell, often in assemblies of 4 to 8 particles. The mean size measured in 13 cells from 9 animals is $150 \pm 8\ \text{\AA}$ (SD = 29, Table 1).

In all parts of the cell there are encountered many irregularly shaped vesicles 300 — $1,000\ \text{\AA}$ in size. They are without exception provided with a single membrane about $40\ \text{\AA}$ wide. The inner structure is homogeneous, mostly with a central lighter part. The vesicles occur, as stated, diffused throughout the cell but, in some isolated cases, a certain regularity was observed. Such vesicles are encountered now and then in the apical parts of the cell where

they may be seen arranged in long rows, parallel to and often between the β -cytomembranes (Fig. 7).

The third component is the homogeneous matrix of the cytoplasm.

DISCUSSION

β -cytomembranes and the cell membrane

The β -cytomembranes appear to have a close relation to the cell membrane so that it is convenient to discuss these two components together. There exists a direct continuity between them at the surface of the cells and, as the opaque components in the β -cytomembranes have exactly the same dimensions (40 Å) as the opaque component in the cell membrane, it may be justified to consider the β -cytomembranes as folds of the cell membrane.

Where two cell membranes are in contact with each other, as is the case at the boundary between two adjacent epithelial cells, there arises the picture of a double membrane which is assumed to be built up of two layers of protein molecules enclosing two double layers of lipid molecules (Sjöstrand and Rhodin, 1953, Sjöstrand and Hanzon, 1954 a). From the total thickness of this double membrane, the total thickness of the cell membrane in the kidney tubules (Sjöstrand and Rhodin, 1953, Rhodin, 1954), in the excretory cells of the pancreas (Sjöstrand and Hanzon, 1954 a), and in the epithelium of the intestine (Zetterqvist, 1956) has been computed to be about 120 Å. With corresponding assumption and basis of computation, the total thickness of the cell membrane in the ciliary epithelium will be about 80 Å.

The β -cytomembranes in the tubular cells of the kidney have been thoroughly analysed by Sjöstrand and Rhodin (1953), and Rhodin (1954). The total thickness of the membrane in these cells is 250 Å which corresponds to two cell membranes in close contact, that is, a condition that is identical with the conditions at the boundary between adjacent, closely packed cells. Thus it is obvious that the β -cytomembranes in the tubular cells of the kidney constitute real duplicates of the cell membrane. The relations between the β -cytomembranes and the cell membranes in the ciliary epithelium, on the other hand, are not so clear. It is true that the thickness (160 Å) of the β -cytomembranes is equivalent to the thickness of two cell membranes in direct contact also in these cells. However, the osmiophobic layer, which would correspond to two double layers of lipid molecules, does not show the same regular thickness as does that of the β -cytomembranes in the kidney. The difference is clearly apparent from the fact that the spreading of the values is substantially greater for the β -cytomembranes as compared with those of

the cell boundary (see Table 1). One explanation might be that the variations in thickness of the β -cytomembranes is an artefact which has arisen during the fixation procedure. The β -cytomembranes in the ciliary epithelium with their position close to the free surface of the cell may be more susceptible to mechanical stresses as compared with the cell membrane at the boundary between adjacent cells, and as compared with the β -cytomembranes in the kidney tubules. This explanation may be questioned because it is difficult to imagine that radically different conditions would exist for the fixation of the cell membrane at the cell boundary and at the β -cytomembranes of the same cells. Therefore, it may be possible that there is a certain difference between the molecular organisation of the cell membrane at the infoldings, which represent the β -cytomembranes, and at the boundaries between closely packed cells.

Therefore, even if the β -cytomembranes in the ciliary epithelium are also to be regarded as duplicates of the cell membrane, their total thickness (160 Å) is appreciably smaller as compared with the β -cytomembranes of the kidney tubules which measure 250 Å. The same relation applies to the constituent components. The opaque layer in the membrane of the ciliary epithelium, as stated above, measures only 40 Å whereas in the kidney tubules it measures 80 Å. This difference in thickness of the opaque layer suggests that the molecular organisation of this constituent is not identical in the two epithelia.

In the kidney, the β -cytomembranes display a very regular topography with division of the basal parts of the cell into digit-like extensions which, for the most part, are filled with mitochondria (Rhodin 1954). There is no such organisation in the ciliary epithelium where the membranes show a very irregular arrangement as regards both their extent in the cell and their direction and positions.

β -cytomembranes are to be found not only in the epithelia of the ciliary body, and of the kidney tubules, but also in the epithelium of choroid plexus (Pease, 1956) though they have not been analysed to any appreciable extent in the last-named epithelium. Thus they constitute a characteristic component in these cells, in which an active transport of water and ions occurs, and they have therefore been assumed to be of importance for this transport (Sjöstrand, 1956 b). More detailed discussion concerning the conceivable rôle of the β -cytomembranes will be given in connection with the experimentally produced changes in the ciliary epithelium (pp. 64 and 65).

The picture observed by Pease (1955 b) in kidney tubules, and which displayed a pronounced enlargement of the middle layers in the membranes has, apart from the large vacuoles and the variations mentioned above not

been observed in the ciliary epithelium in normal conditions. Pease assumes that this enlargement is an expression of a collection of fluid caused by fluid passing from the surrounding cytoplasm through the membranes to the space they delimit. Sjöstrand and Rhodin (1953) and Rhodin (1954), as stated earlier, did not observe similar pictures. There was observed, however, in the ciliary epithelium a pronounced enlargement of the middle layer in fixation injuries and in tissue showing post mortem changes (Fig. 27).

Bargmann *et al.* (1955) observed "perlschnurähnliche Bildungen in Verlauf der Membran" which were taken to be perforations in the membranes. Similar structures have also been observed in the ciliary epithelium (Fig. 7). With high resolution, however, they appear to consist of vesicles lying in rows. No interruptions of the membranes suggesting pore structures were observed. As regards the relation between the vesicles and the β -cytomembranes, attention is directed to page 61.

α -cytomembranes

The analysis of the α -cytomembranes in the ciliary epithelium has shown that the structure of these membranes appears to be entirely in agreement with what was stated by Sjöstrand (1953 f), and Sjöstrand and Hanzon (1954 a). Even as regards the dimensions of both the membranes and particles, the agreement appears to be good. The thickness of 40 Å for the membranes and the particle diameter of 150 Å are the same as what these authors obtained for the membranes in the excretory cells of the pancreas.

The space, which is bounded by the single membranes, is very irregularly shaped in the ciliary epithelium as is the case in the pancreas. In view of the fixation method employed in the present investigation, it should be possible to exclude the possibility that this irregularity is due to post mortem changes.

As regards the content of the space, it has been found in this investigation that it appears homogeneous with a density agreeing with that of the surrounding cytoplasm. No further information regarding its nature and organisation was obtained.

Each pair of membranes appears to constitute an isolated unit. No communication between different pairs was, in fact, observed.

According to several investigators (Porter and Palade, 1952, 1954, Porter, 1953, 1954, Palay and Palade, 1953, 1955, and others), the above-named membranes constitute one of the main components in what is called the "endoplasmic reticulum" forming a network of intercommunicating vesicles and tubules over the whole cell. No such arrangement occurs in the ciliary epithelium. On the contrary, it is a characteristic of this epithelium that the mem-

branes are chiefly encountered in groups in a corner of the cell, most often near its surface. No explanation of this localisation of the membranes in a restricted area could be obtained.

It has been assumed that the α -cytomembranes have an importance for the synthesis of secretion products, particularly proteins (Palade and Porter, 1954, Sjöstrand and Hanzon, 1954 a, Sjöstrand, 1956 b). No synthesis of secretion products similar to that which occurs, for example, in the pancreas appears to take place in the ciliary epithelium. Therefore, the α -cytomembranes in this case do not seem to be directly involved in the production of the secretory product of the ciliary epithelium cells. On the other hand, the α -cytomembranes in the ciliary epithelium may play a rôle in other synthetic processes of the cells.

The Golgi apparatus

The Golgi apparatus does not appear to have been observed with certainty in the ciliary epithelium by light microscopy. A fibrous structure in the vicinity of the nucleus was observed by Stella (1926) among others but his description does not seem to agree with the appearance of the Golgi apparatus in other cells (Kirkmann and Severinghaus, 1938; survey of the Golgi apparatus). The structure encountered in this investigation in the basal parts of the cells displays the same appearance as the Golgi apparatus in other cells examined by means of the electron microscope. There appears, therefore, justification for designating this structure as the Golgi apparatus of the ciliary epithelium.

In the ciliary epithelium, the Golgi apparatus has a definite localisation. Without exception, it is encountered in the cytoplasm between the lower third of the nucleus and the boundary with the pigment epithelium. In all light microscopic examinations made regarding the position of the Golgi apparatus in excretory cells (Kirkmann and Severinghaus, 1938) it was reported located between the nucleus and the excretory pole. Such a polarity is therefore not present in the ciliary epithelium. On the other hand, the position of the Golgi apparatus in these cells in relation to the nucleus is the same as in the kidney (Rhodin, 1954).

Apart from small differences, the Golgi apparatus appears in principle to be similar to structure in most types of cells. The same main components are to be found in different cells: vacuoles, membranes, and granules (Dalton and Felix, 1954, Sjöstrand and Hanzon, 1954 b, c, Rhodin, 1954, Zetterqvist, 1956, and others). The number of membrane pairs is largely the same: 3—6 rows in the ciliary epithelium, 2—5 rows in the pancreas (Sjöstrand and Han-

zon, 1954 c), 4—6 in the tubular cells of the kidney (Rhodin, 1954). Variations in thickness of the membrane also appear to be small. The thickness of the single membranes is similar in the ciliary epithelium (50 Å) as in the pancreas (60 Å), kidney tubules (60 Å), and the intestine (60 Å). As regards the structure of the membrane system there are diverging opinions. According to Palay and Palade (1955) the membranes and the vesicles constitute a continuous system which has been called "agranular reticulum", built up in a manner similar to the "endoplasmic reticulum". According to Lacy (1956) the membrane system in *Patella vulgata* consists of anastomosing double membranes. None of these opinions seems applicable to the Golgi membranes in the ciliary epithelium. In this epithelium it was not possible to observe any communication between two parallel membrane pairs. As stated earlier, the membranes lie in rows arranged in parallel, in each row communication probably occurring between the different membrane pairs but each row constituting an independent unit. The membrane system in the Golgi apparatus of the ciliary epithelium thus appears to be entirely in agreement with the conditions in the pancreas (Sjöstrand and Hanzon, 1954 b, c), the kidney tubules (Rhodin, 1954), and the intestine (Zetterqvist, 1956). Furthermore, it shall be pointed out that, in no case, there were observed any communications between the γ -, the α -, and the β -cytomembranes. Each of these cell components constitutes isolated units.

The vacuoles in the Golgi apparatus, which are bounded by a pair of membranes, are not numerous as compared with, for instance, the pancreas and the intestinal epithelium. In this respect, the ciliary epithelium displays more resemblance to the epithelium of the kidney tubules (Rhodin, 1954), and the corneal epithelium (Sheldon, 1956).

In those cases where vacuoles were observed, they were small as compared with, for instance, those occurring in the intestinal epithelium (Zetterqvist, 1956) where they may have a magnitude of $0,6 \times 1 \mu$. In these last-mentioned cells as also in the liver cells (Fawcett, 1955), there have been observed dense granules in the vacuoles. No structures in the vacuoles in the Golgi apparatus of the ciliary epithelium were observed.

The vesicles between 250 and 800 Å in size, which are grouped in immediate relation to the membranes, appear as regards both size and appearance to correspond to the structures, which in the epithelium of the kidney tubules (Rhodin, 1954) are described as "vacuolar formations", in the liver cells (Fawcett, 1955) as "small vesicles", in the nerve cells (Palay and Palade, 1955) as "circular profiles", and in the intestinal epithelium (Zetterqvist, 1956) as "small vacuoles". On the other hand, they do not appear to correspond

to the small units occurring in the Golgi apparatus of the pancreas (Sjöstrand and Hanzon, 1954 b, c) where they have more the character of granules. On account of their close relations with the γ -cytomembranes, it has been assumed (Sjöstrand and Hanzon, 1954 b, c, Palay and Palade, 1955, Zetterqvist, 1956, and others) that they are derived from the membranes. The present investigation has also brought out a close relation between the units here considered and would therefore appear to support the above assumption. The relations can be so striking that now and again a vesicle may be taken for a membrane pair and vice versa. More over, it has been observed that the vesicles in the Golgi region resemble in shape, size, and appearance the structures occurring freely in the cytoplasm.

The intercellular border

The analysis of the intercellular border has furnished the result that these cell components in the ciliary epithelium are built up in principle in the same manner as in the kidney (Sjöstrand and Rhodin, 1953, Rhodin, 1954), the pancreas (Sjöstrand and Hanzon, 1954 a), the intestine (Zetterqvist, 1956), and other cells investigated.

One characteristic of the ciliary epithelium, however, is the numerously occurring, regularly grouped, and mostly long, narrow processes by which the cells interdigitate into each other. That the intercellular borders may be irregular and folded is known from several types of cells, e. g. epidermis (Ottoson *et al.*, 1953), pancreas (Sjöstrand and Hanzon, 1954 a), liver (Fawcett, 1955), and others, but appreciably less in extent and regularity as compared with the ciliary epithelium. In this very folded area of the cell border in the ciliary epithelium, a thin opaque layer is located in the cytoplasm extending between the folds of the cell membranes. Such a layer occurs between the strongly osmiophilic layers in the myelin sheaths (Sjöstrand, 1953 a), in which there is a thin opaque layer between each such layer. In the ciliary epithelium, however, the thin opaque layer lies only in the cytoplasm of the cells, that is between every second of the opaque cell membrane layers.

Where two cells join at the surface of the epithelium there is thus a highly organised structure, consisting of a membrane system, made up of the cell membranes of the adjacent cells and a thin opaque layer in the cytoplasm (Fig. 12). The folds in this membrane system resemble in principle the β -cytomembranes and as they are located in the same regions of the cells as the β -cytomembranes they may have a similar significance as far as function is concerned.

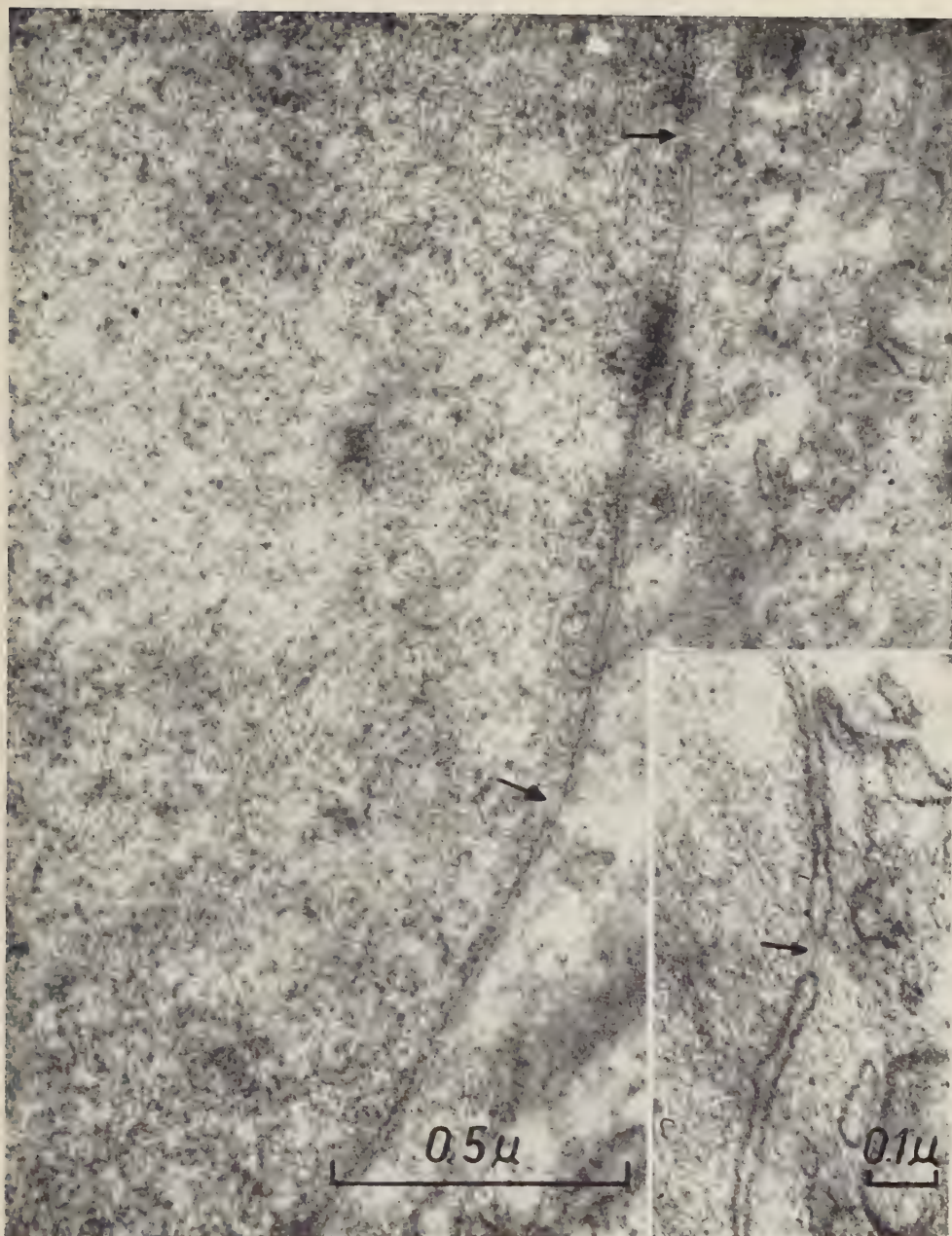


Fig. 26. Part of a nucleus with the nuclear membrane. The inner component of the membrane is thicker than the outer one. All components are very uniform as to thickness. The two osmiophilic layers show a strikingly parallel arrangement. Two "holes" (arrows) are seen in the membrane.
Magnification 77,000 $\times$.

Inset: The "holes" in the nuclear membrane are closed by a thin membrane (arrow).
Magnification 90,000 $\times$.

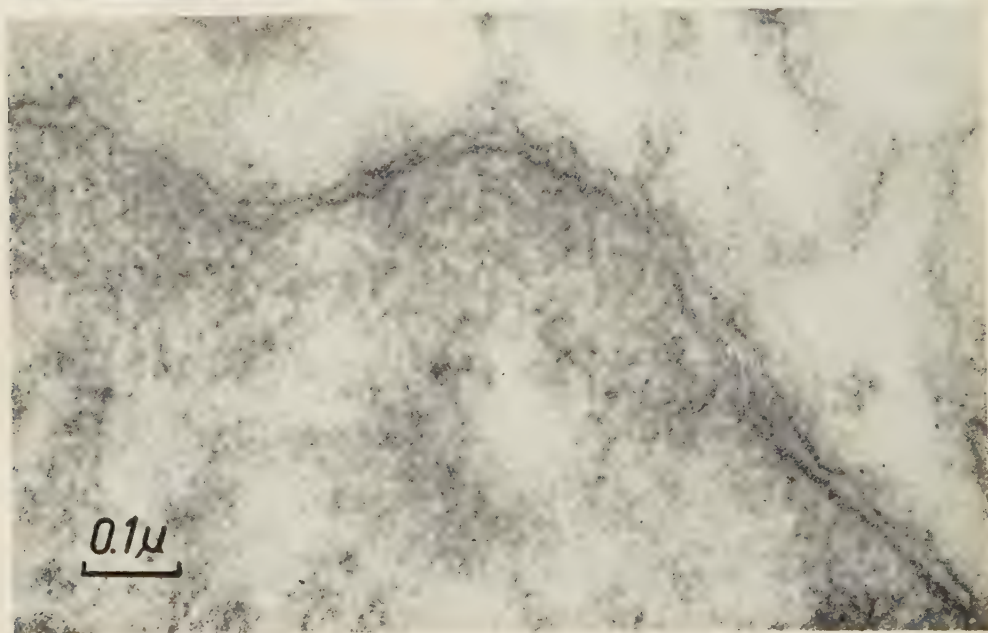
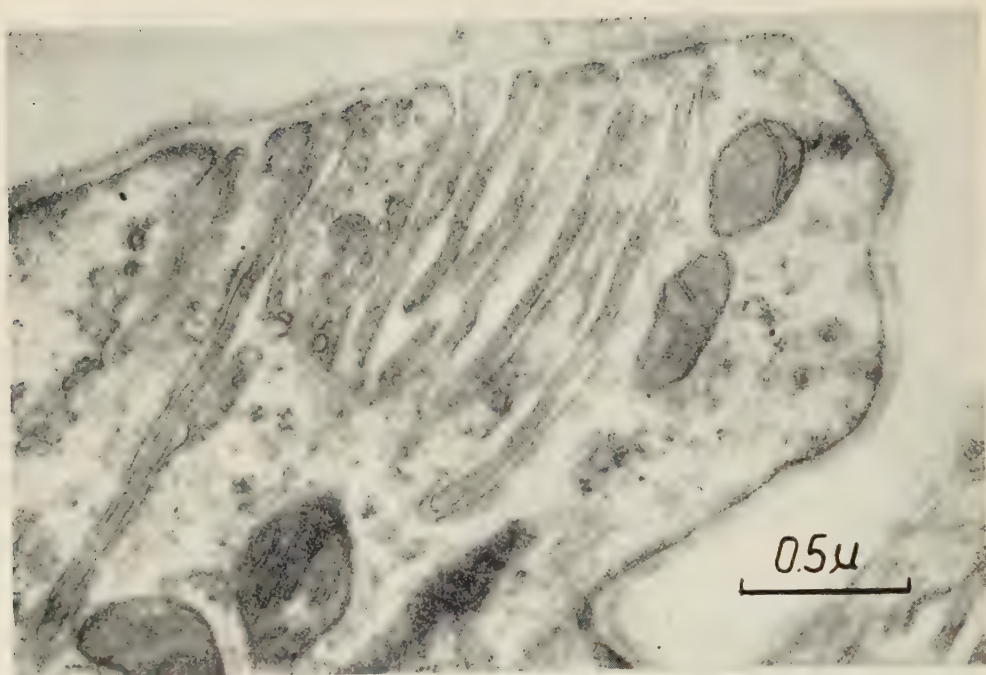


Fig. 27. (The upper picture.) β -cytomembranes from a cell fixed post mortem. The central light space in the membranes is dilated. Compare Figs. 4, 5 and 6. Magnification 42,000 x.

Fig. 28. (The lower picture.) Nuclear membrane from a cell fixed post mortem. The opaque layers are very irregular with large folds and breaks in the continuity. Compare Fig. 26. Magnification 119,000 x.

Terminal bars

The special areas of the cell boundary, in which adjacent cytoplasm is strongly osmiophilic, and which are assumed to correspond to what in light microscopy are called "terminal bars", are numerous represented in the basal parts of the ciliary epithelium. They are built up in the same manner as in the pancreas (Sjöstrand and Hanzon, 1954 a) and the intestinal epithelium (Zetterqvist, 1956) though in these cells as in the epithelium of the kidney tubules (Rhodin, 1954) and others cells they only occur in the apical parts.

It has been assumed (Sjöstrand, 1953 e, Sjöstrand and Rhodin, 1953, Rhodin, 1954, and others) that these "terminal bars" constitute the places of contact between the cells, by means of which increased stability is maintained. Owing to the high organisation which these special areas display, with splitting of the cell membranes into two osmiophilic layers (Zetterqvist, 1956), the question has been raised (Sjöstrand, 1956 b) as to whether the "terminal bars" may not have a special function to fulfil besides a purely mechanical one. As far as the ciliary epithelium is concerned the "terminal bars" are solely confined to the basal parts of the cells, and above all to the boundary line to the pigment epithelium. It is, therefore, doubtful whether the "terminal bars" in these parts would have any mechanical function to fulfil. On the other hand, it is conceivable that a so highly organised structure as the "terminal bars" may represent zones characterised by specially differentiated permeability properties.

The basement membrane

The characterizing as basement membrane of the membrane found in immediate relation but separated from the cell membrane on the free surface of the ciliary epithelium is due solely to its morphology. It has, in fact, the same characteristics as the basement membranes that have been observed in other epithelia (Ottoson *et al*, 1953, Sjöstrand and Rhodin, 1953, and others).

With the light microscope, this membrane would seem to be comprised in the layer that is called internal limiting membrane. It is described (Salzmann, 1912) as a continuous layer which covers the ciliary epithelium and forms processes between the cells. The dimensions of the cell membrane, the basement membrane, and the intermediary layer is far below the resolving power of the light microscope so that the internal limiting membrane is not at uniform structure but is made up of all the above components. What are described as intercellular processes would seem to be nothing but the cell borders as there do not exist any processes of the basement membrane between the cells.

The location of a basement membrane at the surface facing the posterior chamber corresponds, in principle, to what has been observed in the epithelium of the kidney tubules (Sjöstrand and Rhodin, 1953, Rhodin, 1954, Pease, 1955 b, Bargmann, 1955). In the latter cells, a basement membrane is found at the surface of the cells where the β -cytomembranes occur.

With ordinary osmium fixation, the basement membrane seems to be practically homogeneous. No longitudinal striation resembling what Rhodin (1954) observed, or transverse striation such as Bargmann (1955) and Zetterqvist (1956) suggested was observed with any certainty. Phosphotungstic acid increases the contrast in the membrane but it does not contribute to make more details show up as compared with simple osmium fixation. The transverse processes from the basement membrane towards the cell membrane appear to constitute pre-formed connections between the membranes. Their arrangement, which is mostly regular, appears more to support such an opinion. As regards the dimensions, the basement membrane of the ciliary epithelium would seem to agree with corresponding membranes in the epidermis (Ottoson *et al*, 1953) where it measures above 300 Å, and in the intestine (Zetterqvist, 1956) where it measures 270 Å. On the other hand, it differs appreciably from the basement membrane of the kidney tubules which has a thickness of about 800 Å (Sjöstrand and Rhodin, 1954).

Mitochondria

The mitochondria in the ciliary epithelium are small. They have a diameter of only 0.18 μ whereas the corresponding value for the inner segments of the retinal rods is 0.25 μ (Sjöstrand, 1953 e), for the epithelium of the kidney tubules 0.38 μ (Rhodin, 1954), and for the intestinal epithelium 0.27 μ (Zetterqvist, 1956).

They are present in all parts of the cell, and do not show any particular relationship to the β -cytomembranes as in the basal parts of the epithelium of the kidney tubules (Sjöstrand and Rhodin, 1953, Rhodin, 1954, and others), with which the ciliary epithelium in some other respects displays great similarity.

The structural organisation is, with few exceptions, the same as in most of the other cells investigated (Sjöstrand and Rhodin, 1953, Sjöstrand, 1953 f, Rhodin, 1954, Sjöstrand and Hanzon, 1954 a, Zetterqvist, 1956, and others) with an outer limiting membrane made up of three layers, and a number of inner transverse membranes, likewise with three layers. As regards the thickness of the membranes in the mitochondria of the ciliary epithelium, this would seem to be the smallest, yet measured in any cells. Sjöstrand and Rho-

din (1953) state the value of 170 Å for the outer membrane, and 190 Å for the inner membranes in the mitochondria in kidney tubules, Sjöstrand and Hanzon (1954 a) have the values of 140 Å and 170 Å respectively in the pancreas, Zetterqvist (1956) gives the values of 170 Å and 210 Å in the intestine, and Andersson (unpublished) states the values of 170 Å and 220 Å in the muscle cells. The fact that there exists a difference in the dimensions of the inner and outer membranes suggests that the organisation within the membranes is not exactly the same (Sjöstrand, 1956 b). No such difference exists in the mitochondria of the ciliary epithelium.

The variations of the extension of the individual inner membranes in relation to the diameter of the mitochondria suggests that they are in contact with the outer membrane only in some places. They are, therefore, to be taken as being irregularly shaped septa of the type occurring in the excretory cells of the pancreas (Sjöstrand and Hanzon, 1954 a).

According to Palade (1952 b) the inner membranes constitute folds of the outer membrane. In the present investigation there are no proofs that such is the case.

In a few cases, departures from the normal structure were observed. This applies to the arrangement of the inner membranes. Instead of transverse ones, which is normal, there occur one or more large membranes arranged parallel to the long axis of the mitochondrion, and extending from one pole to the other. At the same time, there are found smaller membranes arranged in the regular way. This type of mitochondrion does not appear to have been observed in any other cells investigated. It is hardly probable that they may represent any form of dividing mitochondria. Also the presence of many similarly arranged inner membranes in one and the same mitochondrion contradicts such an opinion. Instead, it would seem justifiable to regard them as a special variant, characteristic of the ciliary epithelium. The fact that variations of the morphology of the mitochondria may occur is shown by an investigation of Rhodin and Dalhamn (1956) who found mitochondria in the ciliated cells of trachea with numerously branched and anastomosing inner membranes.

Another interesting detail in the structure of these mitochondria is that the dimensions of the inner membranes, both in respect of total thickness and the thickness of the constituent component layers, differ in an obvious way from other mitochondria. This circumstance suggests that the molecular organisation is, to some extent, different.

The opaque areas to be found in the mitochondria of some types of cells, such as the tubular cells of the kidney (Palade 1952 b, Sjöstrand and Rhodin, 1953, Rhodin, 1954, and others), the excretory cells of the pancreas (Sjöstrand

and Hanzon, 1954 a), the intestinal epithelium (Zetterqvist, 1956), and others, do not appear to be a component indispensably occurring in the mitochondria of the ciliary epithelium. They were only observed in exceptional cases. The mitochondria in the inner segments of the retinal rods (Sjöstrand, 1953 e), in the corneal epithelium (Sheldon, 1956), and in the ciliated cells of the trachea (Rhodin and Dalhamn, 1956) also lack these structures.

The nucleus

All cell nuclei show a more or less irregular form with wide indentations. The characteristic feature of the nucleus in the ciliary epithelium is that these indentations have the shape of narrow deep clefts, which may have a very large extent both in relation to the circumference of the nucleus and to its diameter.

The other characteristic feature applies to the nuclear membrane. In the ciliary epithelium, as in all other cells investigated, this is made up of three layers, an outer and an inner layer which are osmiophilic, and a less osmiophilic layer interspaced between. The inner layer is found to be appreciably thicker and more osmiophilic than the outer one. The same conditions seem to exist in the ganglion cells (Palay and Palade, 1955) though the dimensions are quite different. These authors give the value of 130 Å for the thickness of the inner component, and 75 Å for that of the outer component. On the other hand no such difference occurs in, e. g., the tubular cells of the kidney (Sjöstrand and Rhodin, 1953, Rhodin, 1954), the intestinal epithelium (Zetterqvist, 1956), the tracheal epithelium (Rhodin and Dalhamn, 1956), or the corneal epithelium (Sheldon, 1956). Fawcett (1955) observed the same arrangement in liver cells but considers that the difference in thickness of the two components is due to an attachment of nuclear particles on the inner layer. No such attachment was observed in the present investigation. Moreover, the nuclear particles are of quite different dimensions. The difference in thickness and in osmiophilia suggests that the two osmiophilic layers in the nuclear membrane of the ciliary epithelium are different regarding their molecular organisation.

Watson's (1954, 1955) interpretation of the nuclear membrane as a flattened vesicle belonging to the "endoplasmic reticulum" does not appear correct. In well fixed material, no communication with adjoining structures was observed, and it should be stressed that in such material both components appear very uniform as to thickness, and with a strikingly parallel arrangement of the two osmiophilic layers. In these respects, the present material differs in a very pronounced way from what has been reported on in earlier investigations.

The uniform thickness of the intermediate, less osmiophilic space of the nuclear membrane may be interpreted as indicating that this space, in fact, consists of a continuous layer connecting the two osmiophilic layers. It certainly is difficult to make these observations agree with the assumption that the nuclear membrane consists of a vesicular structure with the intermediate space representing the lumen of flattened vesicles. On the other hand, with fixation damage, and particularly with post mortem changes it was also observed in the present investigation how in particular the outer component in the membrane was deranged by the formation of large folds and numerous interruptions of the continuity (Fig. 28).

In agreement with a number of other observations "holes" in the nuclear membrane of the ciliary epithelium were observed (Hartman, 1953, 1954, Afzelius, 1955 a, Dawson *et al*, 1955, Palay and Palade, 1955, Zetterqvist, 1956, and others). However, no ring formations corresponding to these "holes" were found on tangential sections. These "holes" in the nuclear membrane occur very sparsely as compared with other cells, e. g., sea-urchin eggs (Afzelius, 1955 a, ganglion cells (Watson, 1954, 1955, Dawson *et al*, 1955), and others. In the present investigation it has been shown, for the first time in mammalian tissue, that the "holes" in the nuclear membrane are bridged over by a thin membrane similar to what is the case in sea urchin eggs (Afzelius, 1955 a). According to André (1956), this membrane in the oocyte of the spider is only due to the thickness of the sections. The thin line observed extending across the "hole" would be due to the fact that part of the edge around the "hole" was included in the section. This explanation seems to be entirely erroneous. It is, indeed, only on the thinnest sections with a direction of the section that is exactly at right angles to the nuclear membrane that a membrane can be observed over the "holes". This membrane is of uniform thickness, and thinner than the osmiophilic components of the rest of the nuclear membrane. In thick sections, the contrast conditions are far too unfavourable for the membrane to appear clearly. Moreover, it is impossible with André's interpretation to obtain such regular u-shaped, and distinctly appearing connections between the two osmiophilic components in the nuclear membrane at the "holes". It would therefore appear incorrect to regard these as pores or fenestræ (Palay and Palade, 1953, Watson, 1955, and others), by means of which an open communication would exist between the cytoplasm and the nuclear substance, as there is no evidence to support such a conclusion in this material.

The investigation of the nuclear substance and the nucleolus did not furnish any further information regarding the organisation of these structures and their arrangement beyond what was known from earlier investigations (Rho-

din, 1954, Sjöstrand and Hanzon, 1954 a, Fawcett, 1955, Palay and Palade, 1955, Zetterqvist, 1956, and others).

Large granules

The large granules represented in Fig. 24 would seem to correspond to what Zetterqvist (1956) in the intestinal epithelium called a vacuole-containing body, which has also been observed in the tracheal epithelium (Rhodin and Dalhamn, 1956). In the present investigation, it has been possible to show that the bounding membrane is made up of three components.

The other large granules (Fig. 25) with very strongly osmiophilic grains do not show any high degree of organisation as compared with the osmiophilic granules in the kidney tubules (Rhodin, 1954), or in the tracheal epithelium (Rhodin and Dalhamn, 1956).

Large spherical bodies

The remaining large cellular component (Figs. 16 and 17), the large spherical body was analysed in far too small a number of cases to make it possible to judge about its structural organisation in a definite way. The double membrane surrounding the spherical body appears identical with the β -cytomembranes as regards dimensions and structure and, judging by observations so far made, the spherical bodies are located in the parts of the cells where these membranes occur. It may, therefore, be supposed that it is merely a question of β -cytomembranes which have received an isolated position owing to the direction of the section. On the other hand, such isolated loops of β -cytomembranes occur fairly often but the structure surrounded by the loops do not show any dissimilarities compared with the cytoplasm. The inner structure of the spherical bodies, however, is quite different, particularly owing to collections of granules of different sizes, the largest of which do not occur freely in the cytoplasm. Moreover, the ground substance is less dense than that of the cytoplasm. There are several factors, therefore, which suggest that the spherical bodies are independent units, and as far as can be judged they are specific for the ciliary epithelium as they have not been observed in any other cells investigated.

The ground substance of the cytoplasm

The small components in the cytoplasm consisting both of particles and of vesicles, which have been observed in this investigation of the non-pigmented

cells in the ciliary epithelium, correspond to those occurring in other cells. A general occurrence of osmiophilic particles in the cytoplasm, 80—300 Å in size, was demonstrated by Palade (1953 b, 1955). Particles and vesicles have been demonstrated in the inner segments of the retinal rods (Sjöstrand, 1953 e), in the tubular cells of the kidney (Sjöstrand and Rhodin, 1953, Rhodin, 1954), in the intestinal epithelium (Zetterqvist, 1956), and in other cells.

The particles occurring freely in the cytoplasm in the size of 150 Å are similar in appearance and shape to those occurring attached to the α -cytomembranes and appear to be identical with similar units in other cells.

There has been observed by light microscopy in the ciliary epithelium (Mawas, 1910, Stella, 1926) an abundance of strongly light refracting grains and vacuoles near the cell surface. It is probable that these are identical with the vesicles observed in the present investigation.

The occurrence of vesicles in the ciliary epithelium, especially in the vicinity of the free surface of the cells, has been considered as constituting evidence that the cells participate actively in the production of the aqueous humour (Seidel, 1920, and others). According to Palade (1953 c), the vesicles in the endothelial cells of blood capillaries represent a transfer of fluid through the cells by means of pinocytosis. Such an assumption would well agree with the conditions in the ciliary body where an abundant fluid flow takes place from the blood to the posterior chamber. As, however, when dealing with static pictures it is impossible to decide whether and in what direction these vesicles move, a direct evidence is lacking for this assumption.

The relation between the vesicles discussed here, and the vesicles in the Golgi apparatus is not clear. Morphologically they are alike but no evidence has been presented to prove that the vesicles are derived from the Golgi apparatus.

CHANGES IN THE STRUCTURE AFTER INJECTION OF DIAMOX

INTRODUCTION

For some years Diamox (acetazoleamid) has been employed as a tension reducing agent in glaucoma. Favourable results were reported in 1954 (Becker 1954 a, b) in patients with glaucoma after peroral doses of between 500 and 1,000 mg. The maximum fall was obtained after 3—5 hours, after which the tension rose, returning to the original level in 8—12 hours. Tonographic examinations made at the same time showed that the outflow of aqueous humour was unchanged and it was therefore assumed that the tension lowering effect was due to an inhibition of the aqueous humour production. Further support for the assumption was obtained by experimental investigations on rabbits and dogs (Becker 1955 a, b, Falbriard *et al.* 1955, Becker and Constant 1956, and others). The nature of the inhibition has not been made clear. The possibility has been excluded, however, (Becker 1955 a) that the diuretic action of Diamox may be the cause. It has been assumed that the primary cause may be a localized inhibition of carbonic anhydrase in the ciliary epithelium which produces aqueous humour. This enzyme is present in the ciliary body (Wistrand 1951), and it has been shown (Becker 1955 b) that after delivery of Diamox the bicarbonate content of the aqueous humour in the posterior chamber decreases in parallel with the fall in the intra-ocular tension. At the same time the bicarbonate concentration in the blood remains constant or almost constant, depending on whether the animal was nephrectomized or not. By means of the change in the bicarbonate content Becker (1955 b) was able to calculate that the production of the aqueous humour is inhibited by Diamox to the extent of about 60 %. Finally Wistrand (1957) has shown that Diamox has a local action on the intraocular tension.

MATERIAL AND METHODS

Altogether 27 rabbits were used for the experiments with Diamox and control experiments. The weight of the rabbits varied between 2.0 kg and 3.3 kg. Both albino and pigmented animals occurred. The distribution of sexes was approximately equal. In one group (group 7) nephrectomy was not

performed, on the other groups nephrectomy was performed between 15 and 20 hours before the start of the experiment. The nephrectomy was performed by careful ligation of both efferent and afferent blood vessels and of the ureter.

The sodium salt of Diamox¹⁾ was used by injection into a vein of the ear. The dosage for the non-nephrectomized animals was 2 injections of 100 mg per kg bodyweight of a 10 % aqueous solution at an interval of 15 min. For the nephrectomized animals the dosage was 10 mg per kg bodyweight of a 1 % aqueous solution in a single injection.

In the control experiments, Diazil (sulfadiminnatrium) was substituted for and used in the same manner as Diamox.

The intraocular tension was recorded for both eyes, using the Schiötz weight-tonometer, loaded with 5.5 g. The tension was measured both before the start of the experiment and in the course of the experiment. The tonometer deflection was converted to mm Schiötz according to the calibration curves applying to the tonometer used.

The fixation of the material and further treatment for electron microscopy was the same as applied to the normal material reported earlier (pp. 10—11).

The experimental animals were divided into groups based on the various experiments (Table 2).

Group	Time in min. after the injection	Dosage in mg. per kg body weight	Number of animals	
	Diazil — treated animals			
1	15	10	5	Nephrectomy
2	30	10	3	"
3	60	10	1	"
4	120	10	1	"
	Diamox — treated animals			
5	15	10	5	Nephrectomy
6	30	10	3	"
7	30	2×100	3	No Nephrectomy
8	60	10	3	Nephrectomy
9	120	10	3	"

Table 2: The groups of the experimental animals.

¹⁾ Supplied through the courtesy of Kemi-Intressen Aktiebolag, Stockholm, Representative of the Lederle Laboratories Division, American Cyanamid Company, New York.

RESULTS

F u n c t i o n a l c h a n g e s

In the 12 animals with which the experiment proceeded for 30 minutes or longer from the injection of Diamox, there was obtained a maximum tension fall varying between 9 and 20 mm Schiötz. In 6 animals the tension fall was

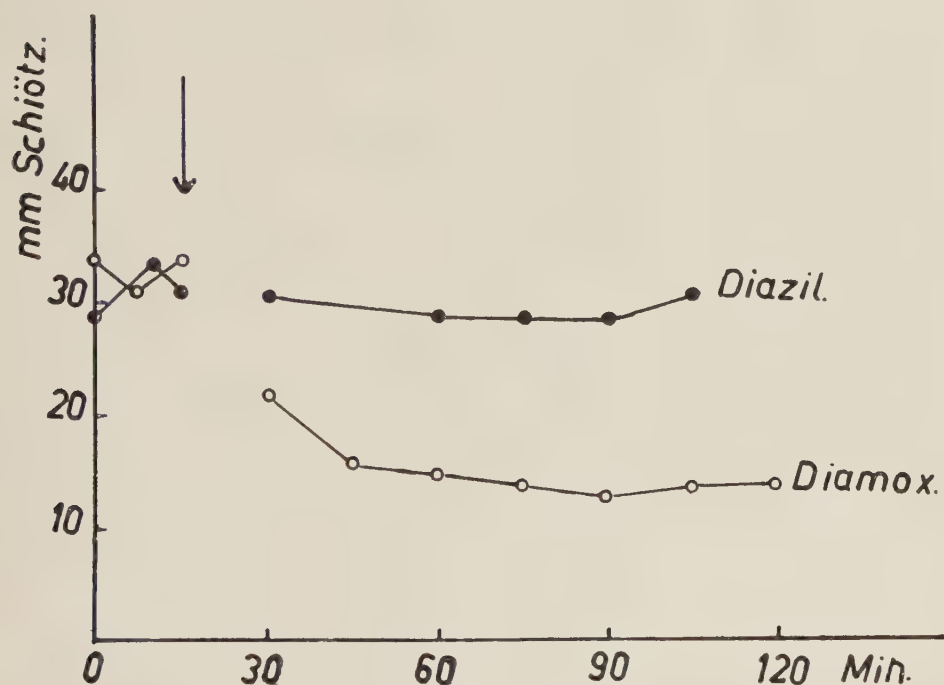
Group	Animal No	Intraocular tension before the injection. Mm Schiötz	Intraocular tension at the end of the experiment. Mm Schiötz
1	98	29	29
	100	29	29
	102	27	22
	103	27	29
	107	22	22
2	91	25	29
	101	29	32
	106	29	25
3	95	29	22
4	87	29	31
5	96	32	23
	97	29	19
	99	29	22
	104	34	19
	105	32	22
6	88	23	11
	89	29	10
	90	34	17
7	74	25	14
	75	29	14
	76	29	12
8	92	23	13
	93	19	11
	94	25	14
9	84	29	11
	85	31	11
	86	34	14

Table 3: Intraocular tension before and after treatment.

greater than 15 mm, in 5 animals it was between 10 and 15 mm and in one animal it was less than 10 mm (Table 3).

From Textfigure 3 it may be seen how the tension fall proceeds after a single injection of Diamox. In about 30 min. the maximum fall has developed and the tension stands during the remainder of the experiment at a constant low level. Textfigure 3 also shows tension conditions in a control animal which received Diazil instead of Diamox. It was not possible to record any certain tension fall after Diazil in any single case.

In the 5 animals in which the experiment proceeded 15 min. after injection of Diamox the tension fall varied between 7 and 15 mm Schiötz (Table 3).



Textfig. 3. Intraocular tension of two animals after injection (arrow) of 10 mg Diamox and 10 mg Diazil per kg body weight respectively. Both animals were nephrectomized 20 hours before the injection.

Ultrastructural changes

In the following account, only those cell components which undergo pronounced change after injection of Diamox will be dealt with.

Mitochondria

No change in the distribution of mitochondria in the cell or their number compared with normal animals or the Diazil-treated animals appears to occur after injection of Diamox. On the other hand, there sets in rapidly after the

Group	Animal No	The mean width in μ of the mitochondria for each animal	SD	The mean with in μ of the mitochondria for each group	SD
Normal				$0,18 \pm 0,005$	0,026
1	98	$0,19 \pm 0,006$	0,020	$0,18 \pm 0,002$	0,005
	100	$0,18 \pm 0,005$	0,017		
	102	$0,18 \pm 0,004$	0,014		
	103	$0,18 \pm 0,005$	0,017		
	107	$0,18 \pm 0,009$	0,028		
2	91	$0,17 \pm 0,003$	0,010	$0,19 \pm 0,009$	0,016
	101	$0,19 \pm 0,005$	0,017		
	106	$0,20 \pm 0,006$	0,020		
3	95	$0,16 \pm 0,005$	0,017	$0,16 \pm 0,005$	0,017
4	87	$0,19 \pm 0,003$	0,010	$0,19 \pm 0,003$	0,010
5	96	$0,26 \pm 0,006$	0,020	$0,26 \pm 0,008$	0,017
	97	$0,29 \pm 0,005$	0,017		
	99	$0,26 \pm 0,006$	0,017		
	104	$0,26 \pm 0,001$	0,004		
	105	$0,24 \pm 0,006$	0,020		
6	88	$0,25 \pm 0,006$	0,020	$0,25 \pm 0,004$	0,007
	89	$0,25 \pm 0,005$	0,016		
	90	$0,24 \pm 0,008$	0,024		
7	74	$0,26 \pm 0,010$	0,030	$0,25 \pm 0,013$	0,023
	75	$0,26 \pm 0,010$	0,028		
	76	$0,22 \pm 0,005$	0,014		
8	92	$0,27 \pm 0,003$	0,010	$0,25 \pm 0,016$	0,026
	93	$0,26 \pm 0,008$	0,026		
	94	$0,22 \pm 0,005$	0,014		
9	84	$0,26 \pm 0,007$	0,023	$0,26 \pm 0,006$	0,010
	85	$0,27 \pm 0,007$	0,023		
	86	$0,25 \pm 0,008$	0,026		

Table 4: The mean width of the mitchondria.

injection a pronounced increase of the width of the mitochondria. This change is fully expressed already 15 minutes after the injection and would appear to be maintained constant during the action of Diamox (Table 4), so that the width of the mitochondria is still the same 120 minutes after the injection. The difference in the mean width of the mitochondria between the different experimental groups may be seen in Table 5, in which the significances are given. It will be noted that between groups 1 and 2 there is no difference whereas a definite difference is present between these groups on the one hand and groups 5, 6, 8 and 9 on the other. Between these last-named groups themselves there is no significant difference. Presented graphically, therefore, this means for the Diamox-treated animals a curve which has an initial minimum and rises rapidly to a constant level, without tendency to return to the initial value during the time the experiment was in progress (Textfigure 4).

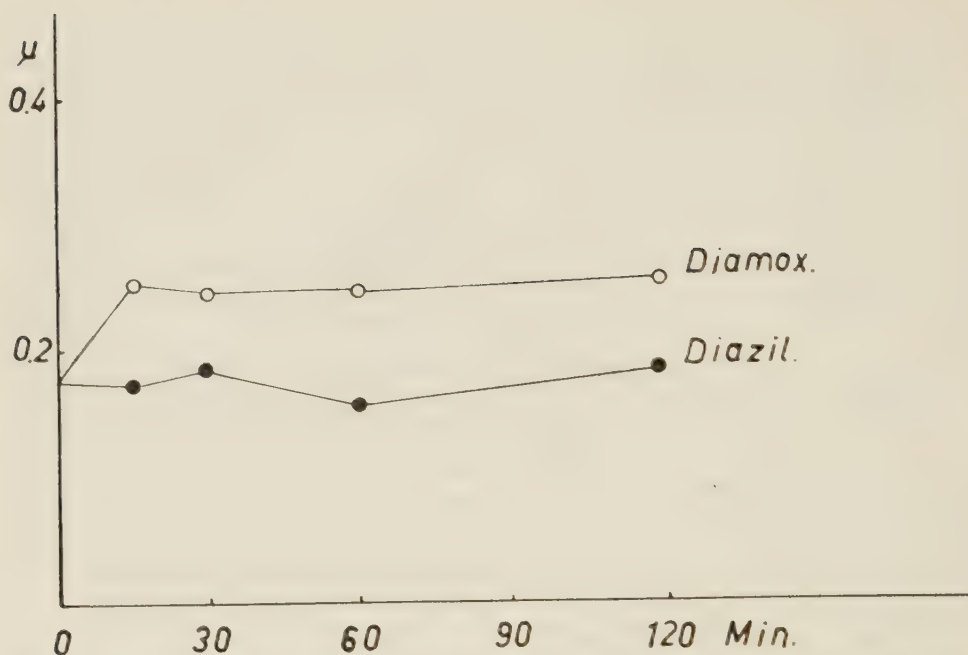
As regards the variations in the width of the mitochondria it may be seen from Table 4 that no difference in variance is present between the Diamox-treated animals when compared with the Diazil-treated animals and the normal animals, neither when it is a question of dispersion between different cells in the animal or between the different animals in the same group.

The changes in the thickness of the mitochondria are entirely due to an increase of their ground substance (Figs. 29, 30, 31, 32). No change occurs of the type recorded in mitochondria with post mortem changes, which are characterised by a loss of the ground substance.

In the great majority of cases there does not occur any change of the ultra-structure of the mitochondria, neither as regards the arrangement or the dimensions of the outer and inner membranes (Fig. 29). Now and again, however, there were observed mitochondria with very short and very irregularly arranged inner membranes (Figs. 31, 32). All the cases so observed were extremely wide mitochondria.

Group	t - values					M
	2	5	6	8	9	
1	0,68°	9,70***	17,88***	6,07***	3,89**	0,18
2		6,05***	6,33***	3,59*	6,94**	0,19
5			1,40°	0,71°	0,17°	0,26
6				0,21°	2,00°	0,25
8					0,61°	0,25
9						0,26

Table 5: The significance of the differences in the mean width of the mitochondria.



Textfig. 4. Diagram of the mean width of the mitochondria after injection of Diamox and Diazil respectively. The zero time represents the normal animals.

The Golgi apparatus

Diamox has no action on the Golgi apparatus in respect to its position in the cell, its composition or the thickness of the membranes comprised in the Golgi apparatus.

In two respects at least there arise characteristic changes in the animals treated with Diamox. This applies to the number of vesicles in the Golgi region and the length of the rows of double membranes.

Tables 6 and 7 present a summary of the analysis made in respect of the number of vesicles. The analysis was made by counting all the vesicles on *one* section through the Golgi region representing *one* cell. Regarding group 5, it should be stated that the figure for the number of vesicles in this group may be too high, due to the fact that these animals display a large amount of vesicles even in other parts of the cell. This uncertainty is not present in of the other groups.

From Table 6 it may be seen that the figures for the number of vesicles are already 15 minutes after the injection of Diamox approximately double (Fig. 33) those in normal and control animals, and after 30 minutes they are almost

Group	Animal No	Number of Golgi apparatus counted	Mean number of vesicles per Golgi apparatus per section for each animal	SD	Mean number of vesicles per Golgi apparatus per section for each group	SD
Normal		31			78 ± 4	13
1	98	10	70 ± 8	25	76 ± 3	7
	100	8	85 ± 3	7		
	102	7	69 ± 13	35		
	103	7	76 ± 13	34		
	107	6	80 ± 12	30		
2	91	4	86 ± 18	35	78 ± 4	11
	101	11	74 ± 11	36		
	106	8	75 ± 12	34		
3	95	7	93 ± 12	32	93 ± 12	32
4	87	7	79 ± 11	31	79 ± 11	31
5	96	6	164 ± 21	51	166 ± 9	20
	97	7	185 ± 16	43		
	99	8	193 ± 8	21		
	104	10	145 ± 17	55		
	105	9	142 ± 14	42		
6	88	13	193 ± 12	44	198 ± 16	28
	89	11	173 ± 11	36		
	90	4	228 ± 40	80		
7	74	6	195 ± 23	54	189 ± 6	11
	75	6	174 ± 16	38		
	76	4	198 ± 25	49		
8	92	5	184 ± 13	33	160 ± 12	21
	93	4	142 ± 7	14		
	94	6	155 ± 15	35		
9	84	6	79 ± 10	24	104 ± 12	36
	85	6	116 ± 15	36		
	86	9	118 ± 7	20		

Table 6: The mean number of Golgi vesicles.

Group	t - values					M
	2	5	6	8	9	
1	0,48°	8,39***	9,84***	8,46***	2,81*	76
2		6,25***	7,24**	6,31**	1,96°	78
5			1,78°	0,33°	3,71**	166
6				1,85°	4,58*	198
8					3,15*	160
9						104

Table 7: The significance of the differences in the mean number of Golgi vesicles.

trebled (Figs. 34, 35). After 60 minutes the number has diminished somewhat and after a further hour the number is still lower. An analysis of the relations between the different groups is given in Table 7. As with the mitochondria there is no difference between groups 1 and 2, whereas a significant difference is present between these groups on the one hand and groups 5, 6, 8 and 9 on the other. An exception is constituted by the difference between group 2 and group 9, where there is a great numerical difference, which is not significant, however. No significant differences exist between groups 5, 6 and 8, but there is a significant difference between these groups and group 9. Thus the changes in the Golgi apparatus after injection of Diamox in this respect may be presented by a curve, with an initial minimum and rising to a maximum in 15 to 60 minutes (groups 5, 6, 8) and which after a further time displays a falling tendency, without returning to the initial value, however during the time the experiment was in progress (Textfig. 5). Numerically the maximum would appear to lie in group 6, i. e. 30 minutes after the injection of Diamox.

The magnitude of the dosage of Diamox in the limits used here, does not seem to have any influence on the number of vesicles. As may be seen from Table 6, the number of vesicles in group 7 (30 minutes after 2 injections of Diamox each of 100 mg per kg bodyweight on non-nephrectomized animals) is the same as in group 6.

The variance in the number of vesicles in the Golgi region is remarkable. As may be seen in Table 6 the dispersion between different cells in one and the same animal is substantially greater in groups 5, 6 and 7 compared with the other groups. Where it is a question of the dispersion between the different animals in one and the same group a similar tendency appears. In groups 5, 6, 8 and 9 the dispersion is obviously greater than in the normal animals and in groups 1 and 2. The great dispersion between the animals in group 9, as also the small dispersion in group 7, are noteworthy.



Fig. 29. *Diamox* 120 minutes. Mitochondria. As compared with Fig. 18 the mitochondria are thicker.
Magnification 65,000 x.



Fig. 34. Diamox 30 minutes. Golgi apparatus. As in Fig. 33 there are several Golgi units (arrows), each consisting of only a few short rows of γ -cytomembranes and many vesicles. Magnification 72,000 $\times$.

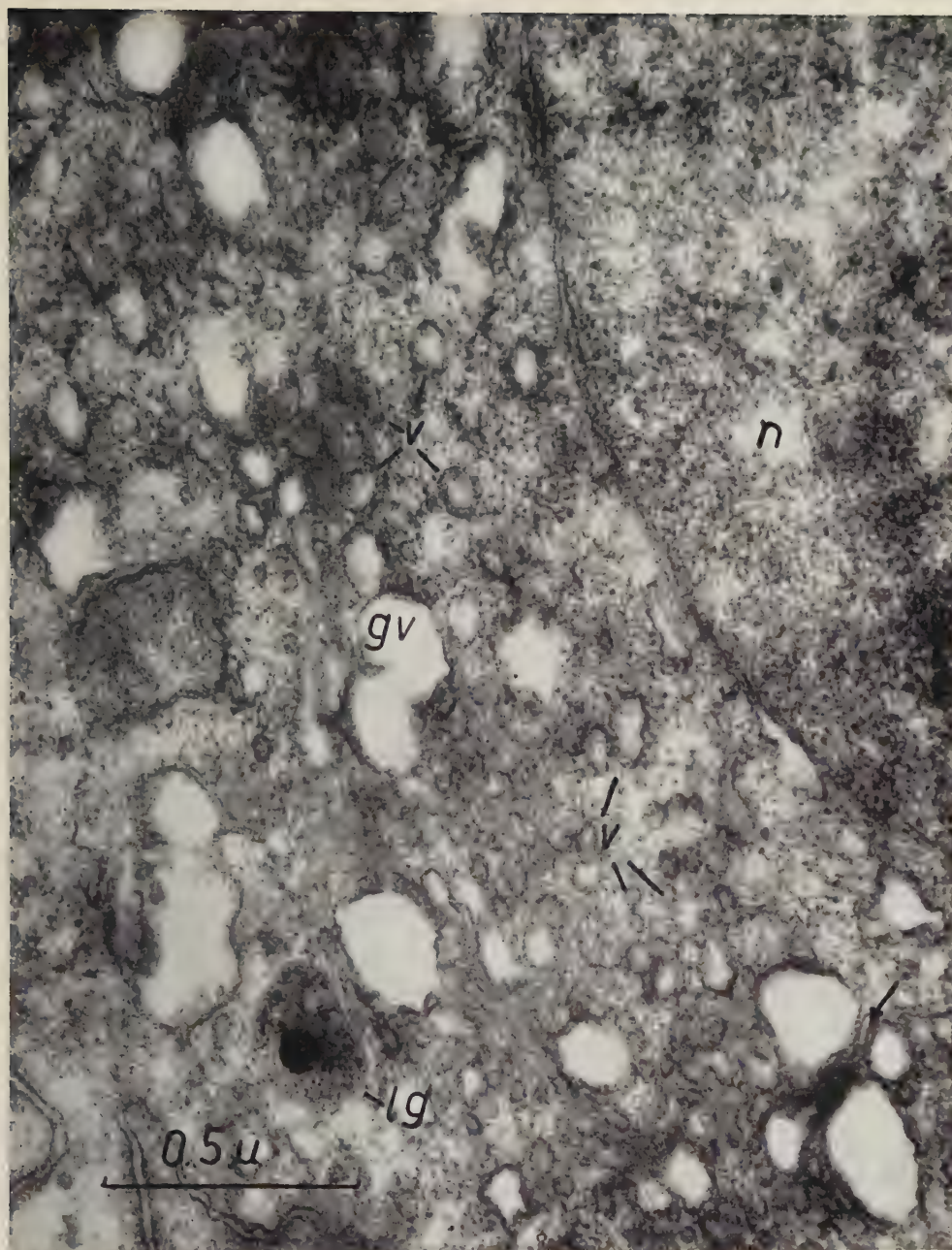


Fig. 35. Diamor 30 minutes. Golgi apparatus. There are only a few r-cytoplasmic membranes (arrow) but several large vacuoles (gv) and many vesicles (v). A part of the nucleus (n) lies to the right. The large granule (lg) is more highly magnified in Fig. 25. Magnification 70,000 x.

The changes in the membrane system were analysed in the first place in respect to length. As described in the chapter on the normal structure (page 19) the separate double membranes are arranged in several parallel rows. What has been measured in this investigation is solely the length of these rows in each Golgi unit. Thus the measurements comprise only a part of the Golgi region, not its whole extent.

The result of this analysis is given in Tables 8 and 9, the latter showing the significances of the differences in the means. There does not exist any significant difference between groups 1 and 2. A significant difference is to be found, however, between group 1 and groups 5, 6, 8 and 9, as also between group 2 and groups 5 and 6. Between groups 5 and 6 there is no difference, but a difference does exist between groups 5 and 6 on the one hand and groups 8 and 9 on the other, thus indicating that groups 5 and 6 represent minimum figures. Between group 8 and group 9 there is no difference, but the figures for both these groups are significantly lower than the figure for group 1. The variation in the length of the membrane system is presented graphically in Textfig. 5. In the control groups (groups 1—4) there are no changes in the length. Fifteen to thirty minutes after injection of Diamox the length is reduced to a minimum (groups 5 and 6). After longer time after the injection (groups 8 and 9) the length is increasing though without returning to the value for the control group.

In respect to the variance of the length this appears to be more or less the same throughout the material, both between different cells in one and the same animal and between different animals in the same group. Nevertheless, the small dispersion in group 7 should be noted, this being the lowest in the whole material.

The length of the rows of membrane pairs has decreased. This may be due to the rows consisting of smaller membranes or possibly the number of such membranes in each row may have decreased.

Finally, as regards the changes in the Golgi region, one condition that is striking should be referred to and that is the number of Golgi units in each cell. As stated earlier, (page 18) in the normal animal there is usually 1 to 2 units, rarely 3 or more per section. In the Diamox-treated animals there often occur 4 or more units (Figs. 33—36). Cells with up to 7 units have been observed in exceptional cases. Table 10 gives a simple statement of this condition.

As may be noted, the greatest number of Golgi units is to be found in groups 5, 6 and 7, while group 9 displays the smallest number. The material is too small for thorough analysis to permit a significant conclusion.

Group	Animal No	Number of rows measured	The mean length in μ of the rows of membrane pairs for each animal	SD	The mean length in μ of the rows of membrane pairs for each group	SD
Normal		57			$0,66 \pm 0,054$	0,201
1	98	23	$0,64 \pm 0,055$	0,265	$0,63 \pm 0,036$	0,081
	100	16	$0,62 \pm 0,037$	0,149		
	102	12	$0,57 \pm 0,075$	0,258		
	103	7	$0,65 \pm 0,046$	0,122		
	107	16	$0,67 \pm 0,037$	0,149		
2	91	10	$0,63 \pm 0,042$	0,133	$0,58 \pm 0,033$	0,058
	101	19	$0,60 \pm 0,046$	0,199		
	106	12	$0,52 \pm 0,049$	0,169		
3	95	8	$0,60 \pm 0,064$	0,202	$0,60 \pm 0,064$	0,202
4	87	10	$0,59 \pm 0,033$	0,095	$0,59 \pm 0,033$	0,095
5	96	15	$0,46 \pm 0,048$	0,189	$0,41 \pm 0,046$	0,104
	97	16	$0,39 \pm 0,038$	0,148		
	99	19	$0,40 \pm 0,019$	0,083		
	104	13	$0,40 \pm 0,029$	0,106		
	105	21	$0,40 \pm 0,039$	0,177		
6	88	24	$0,43 \pm 0,027$	0,132	$0,40 \pm 0,021$	0,036
	89	21	$0,41 \pm 0,024$	0,112		
	90	19	$0,36 \pm 0,023$	0,101		
7	74	17	$0,40 \pm 0,035$	0,145	$0,39 \pm 0,010$	0,017
	75	7	$0,39 \pm 0,075$	0,198		
	76	8	$0,37 \pm 0,046$	0,131		
8	92	14	$0,53 \pm 0,039$	0,147	$0,52 \pm 0,026$	0,046
	93	12	$0,47 \pm 0,033$	0,115		
	94	7	$0,56 \pm 0,046$	0,122		
9	84	14	$0,54 \pm 0,037$	0,140	$0,55 \pm 0,032$	0,055
	85	18	$0,61 \pm 0,045$	0,191		
	86	8	$0,50 \pm 0,014$	0,040		

Table 8: The mean length of the rows of Golgi membrane pairs.

Group	t - values					M
	2	5	6	8	9	
1	1,42°	10,37***	8,42***	3,69*	2,45*	0,63
2		5,91**	4,71**	1,50°	0,72°	0,58
5			0,44°	4,29**	4,84**	0,41
6				3,57*	3,93*	0,40
8					0,72°	0,52
9						0,55

Table 9: The significance of the differences in the mean length of the rows of the Golgi membrane pairs.

Group	Number of Golgi units per cell per group
Normal	2,0
1	2,1
2	2,3
5	4,4
6	3,0
7	3,0
8	2,6
9	1,7

Table 10: Number of Golgi units per cell.

The cytoplasm

The third marked change after the injection of Diamox makes its appearance in the cytoplasm. In animals fixed 15 min. after injection, a considerable assembly of vesicles is seen in the cytoplasm (Figs. 37, 38). An abundant but reduced quantity is still present 30 min. after injection, but after longer times it appears on a rough estimate as if their quantity was the same as for the control animals.

The vesicles have a width of between 200 and 700 Å and a length of between 200 and 2000 Å. Without exception, they are surrounded by a single membrane about 40 Å wide. The structure bounded by this appears quite homogeneous, with a central lighter part.

A thorough analysis of the occurrence of the vesicles in the cells was undertaken, both as regards their general frequency and as regards the frequency in different zones of the cell. These zones were selected exclusively in the apical parts of the cell. One zone, designated the 1 μ zone, comprises an area with a depth of 1 μ from the cell membrane. The other zone, designated the 2—3 μ zone, stretches from the 1 μ zone to a depth of 3 μ from the cell membrane. The reason that no analysis was made of the middle and basal parts of the cells is that in these parts there are the cell nucleus and the Golgi apparatus respectively, which both make a random selected determination impossible. Between 7 and 15 cells were examined in each animal. In each zone, the vesicles were counted on four separate fields, each comprising an area 0.05 μ^2 in magnitude. The total surface for each zone in which the vesicles were counted was thus so large that it was possible to have in it minimally 1—2 vesicles and maximally 20—25 vesicles. The advantage with this was on the one hand that one did not get Poisson divisions and on the other hand that the accuracy in the estimation of the individual cell was not unreasonably great compared with the comparatively small groups of animals.

As a gauge of the occurrence of vesicles in the respective zones it is convenient to give the frequency of vesicles per field. By counting four such fields in each zone, a fairly large material has been obtained. As the fields examined in each zone were selected at random it happened now and again that other components than those we were concerned with fell within the field in question. If the field was filled up to the extent of half or more in this way, it was rejected. The result has been that in some zones fewer than four fields were examined. The gaps would appear to be distributed fairly at random in the whole material, as regards both animals and groups. When counting the number of vesicles in a field, the vesicles sometimes did not fall to their full extent in the field. The principle followed was that if any part of a vesicle fell in the field it was counted.

The result of the analysis is presented in Tables 11—13. Table 11 gives only the frequency for the two zones together and the difference in frequency between the 1 μ zone and the 2—3 μ zone. Table 12 gives the significance of the difference in frequency between the various groups and Table 13 the significance of the difference in frequency between the 1 μ zone and the 2—3 μ zone in each group.

In the control groups 1 and 2 there exists a frequency of 0.5 and 0.7 vesicles respectively. The difference in frequency is not significant. Groups 3 and 9, each represented by one animal, show a frequency which differs numerically but slightly from these figures, and one may therefore assume that all the control animals show the same frequency. Thus no change in the frequency of vesicles with respect to length of treatment would appear to exist.

Following the injection of Diamox, there occurs as may be seen from Table 11 a pronounced rise in the frequency of cytoplasmic vesicles which is most marked in groups 5 and 6. All the groups treated with Diamox have significantly higher frequency than group 1 (Table 12). Group 5 displays a significantly higher frequency than group 6, and both these groups have a significantly greater frequency than groups 8 and 9. There is no apparent difference

Group	Animal No	Mean frequency of vesicles within 1μ zone + $2-3\mu$ zone			Difference in the mean frequency of vesicles between 1μ zone and $2-3\mu$ zone		
		Animal	Group	SD	Animal	Group	SD
1	98	0,77	$0,51 \pm 0,076$	0,169	— 0,03	$+ 0,12 \pm 0,058$	0,129
	100	0,42			+ 0,14		
	102	0,60			+ 0,12		
	103	0,40			+ 0,67		
	107	0,36			+ 0,32		
2	91	0,75	$0,67 \pm 0,065$	0,112	+ 0,14	$+ 0,25 \pm 0,104$	0,180
	101	0,72			+ 0,45		
	106	0,54			+ 0,14		
1 + 2						$+ 0,17 \pm 0,053$	
3	95	0,61	$2,85 \pm 0,273$	0,610	+ 0,02	$- 0,45 \pm 0,625$	1,398
4	87	0,66			+ 0,15		
	96	3,14			+ 0,54		
5	97	2,51			— 0,43		
	99	3,24			+ 0,27		
6	104	3,39			— 2,87	$+ 0,03 \pm 0,326$	0,564
	105	1,94			+ 0,34		
	88	2,17	$1,90 \pm 0,173$	0,299	+ 0,33		
	89	1,94			+ 0,37	$+ 0,10 \pm 0,240$	0,415
	90	1,58			— 0,62		
8	92	1,12	$0,94 \pm 0,151$	0,262	+ 0,40	$+ 0,33 \pm 0,153$	0,265
	93	0,64			— 0,37		
	94	1,06			+ 0,26		
9	84	0,70	$0,97 \pm 0,137$	0,237	+ 0,03		
	85	1,12			+ 0,46	$+ 0,33 \pm 0,153$	0,265
	86	1,11			+ 0,51		

Table 11: The mean frequency of vesicles and the difference in the mean frequency between 1μ zone and $2-3\mu$ zone.

between the last two groups. Table 11 also shows that there exists a marked difference between the Diazil-treated animals (groups 1 and 2) and those treated with Diamox (groups 5, 6, 8 and 9), even to the extent that the variations in frequency between different animals are appreciably greater in the

Group	t - values					M
	2	5	6	8	9	
1	1,42°	8,25***	8,55***	2,87*	3,26*	0,51
2		5,93**	6,66**	1,64°	1,99°	0,67
5			2,47*	5,01**	4,97**	2,85
6				4,15*	4,19*	1,90
8					0,16°	0,94
9						0,97

Table 12: The significance of the differences in the mean frequency of vesicles.

Diamox-treated animals. As may be seen, the size of the dispersion in groups 1 and 2 is quite different compared with groups 5, 6, 8 and 9. The great dispersion in group 5 is striking.

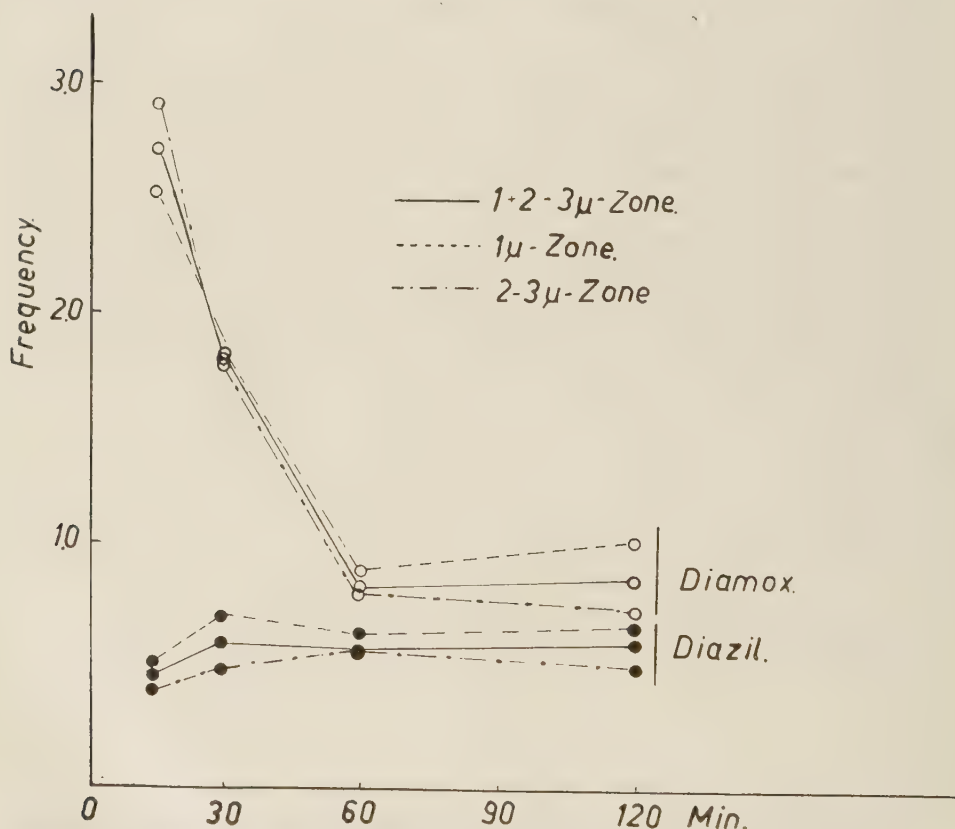
When it is a question of the frequency in the different zones reckoned separately (Table 11), it is found that for groups 1 and 2 the 1 μ zone has a frequency that is about 0.2 vesicles greater than that of the 2—3 μ zone. This difference, however, is not significant for either of the groups (Table 13) but for the two groups reckoned together the difference is significant. The same tendency is repeated in groups 3 and 4. Where the groups 5, 6, 8 and 9 are concerned there does not appear any difference in this respect either, but in this case the conditions appear different. In group 5 the 2—3 μ zone shows numerically greater frequency of vesicles than the 1 μ zone, but this is due for the most part to animal 104 and to a certain extent to animal 97. Even in

Group	Difference in the mean frequency	t-values
1	+ 0,12	2,15°
2	+ 0,25	2,36°
1 + 2	+ 0,17	3,18*
5	— 0,45	0,72°
6	+ 0,03	0,09°
8	+ 0,10	0,41°
9	+ 0,33	2,18°

Table 13: The significance of the difference in the mean frequency of vesicles between 1 μ zone and 2—3 μ zone.

groups 6 and 8 individual animals (90 and 93) show numerically a greater frequency for the 2—3 μ zone. Again in group 9 the 1 μ zone in all animals has a greater frequency. Examining the above-cited animals (90, 93, 97 and 104) more closely it will be found that in all the cells examined or the greater part of them the 2—3 μ zone has a greater frequency of vesicles. (The condition is clearly seen on Fig. 37 representing animal 104. In a small area next to the surface, vesicles are almost entirely lacking.) Thus it is not a question of a chance figure for isolated cells but it is a clear tendency in all the cells of these animals. For the other animals in all groups the distribution of the cells was such that the number of cells in each animal which gave a higher frequency of cytoplasm vesicles in the 1 μ zone was slightly greater than the number of cells where the 2—3 μ zone had higher frequency.

The results of the examination made are presented in Textfigure 6.



Textfig. 6. Diagram the mean frequency of vesicles in the cytoplasm after injection of Diamox and Diazil respectively.



Fig. 37. Diamox 15 minutes. The cytoplasm is filled with vesicles. Within a zone close to the cell surface the vesicles are almost lacking. Compare Figs. 4, 9 and 18. Magnification 59,000 x.

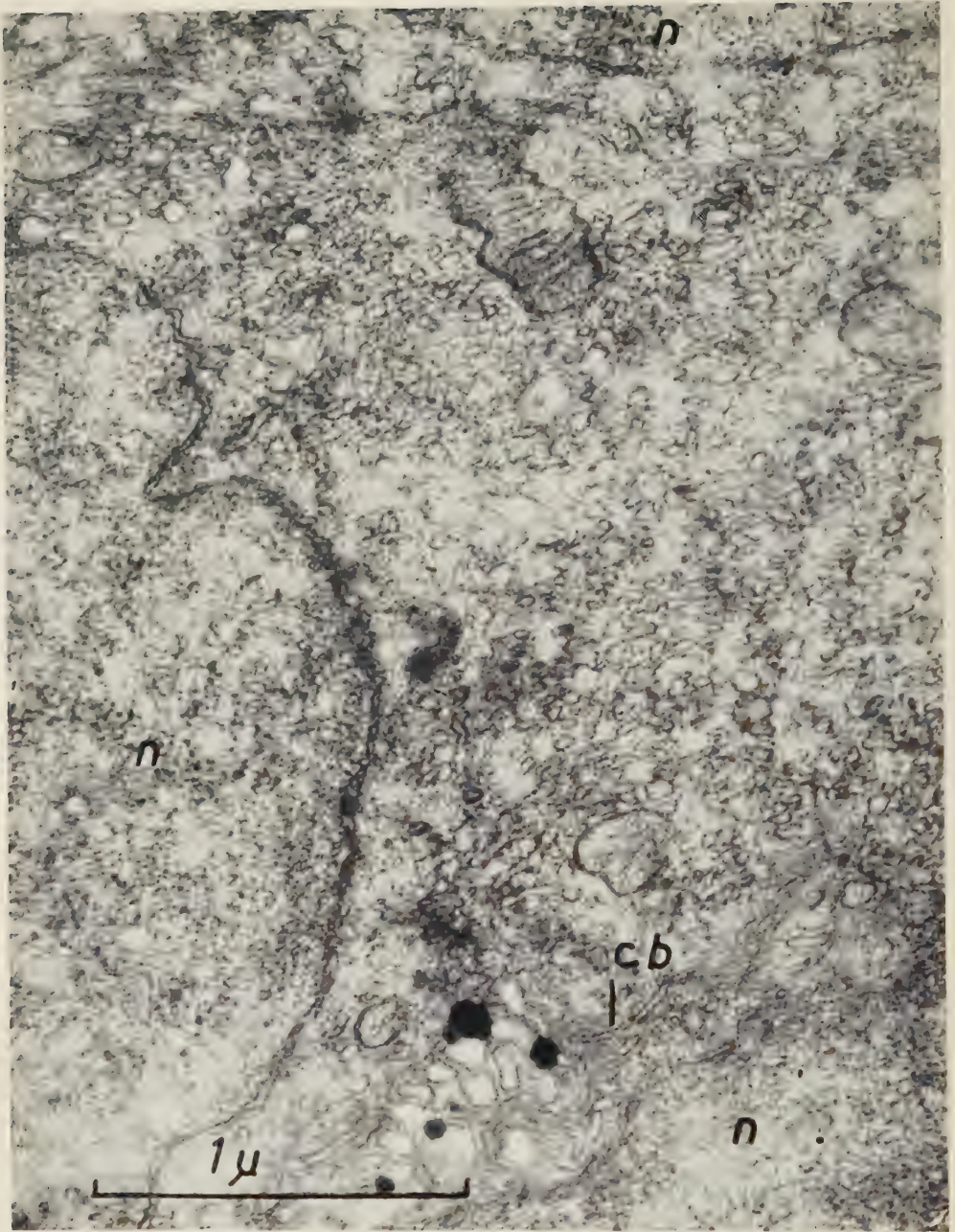


Fig. 38. Diamox 15 minutes. The cytoplasm between the nuclei (n) and the cell border (cb) is filled with vesicles.
Magnification 44,000 $\times$.

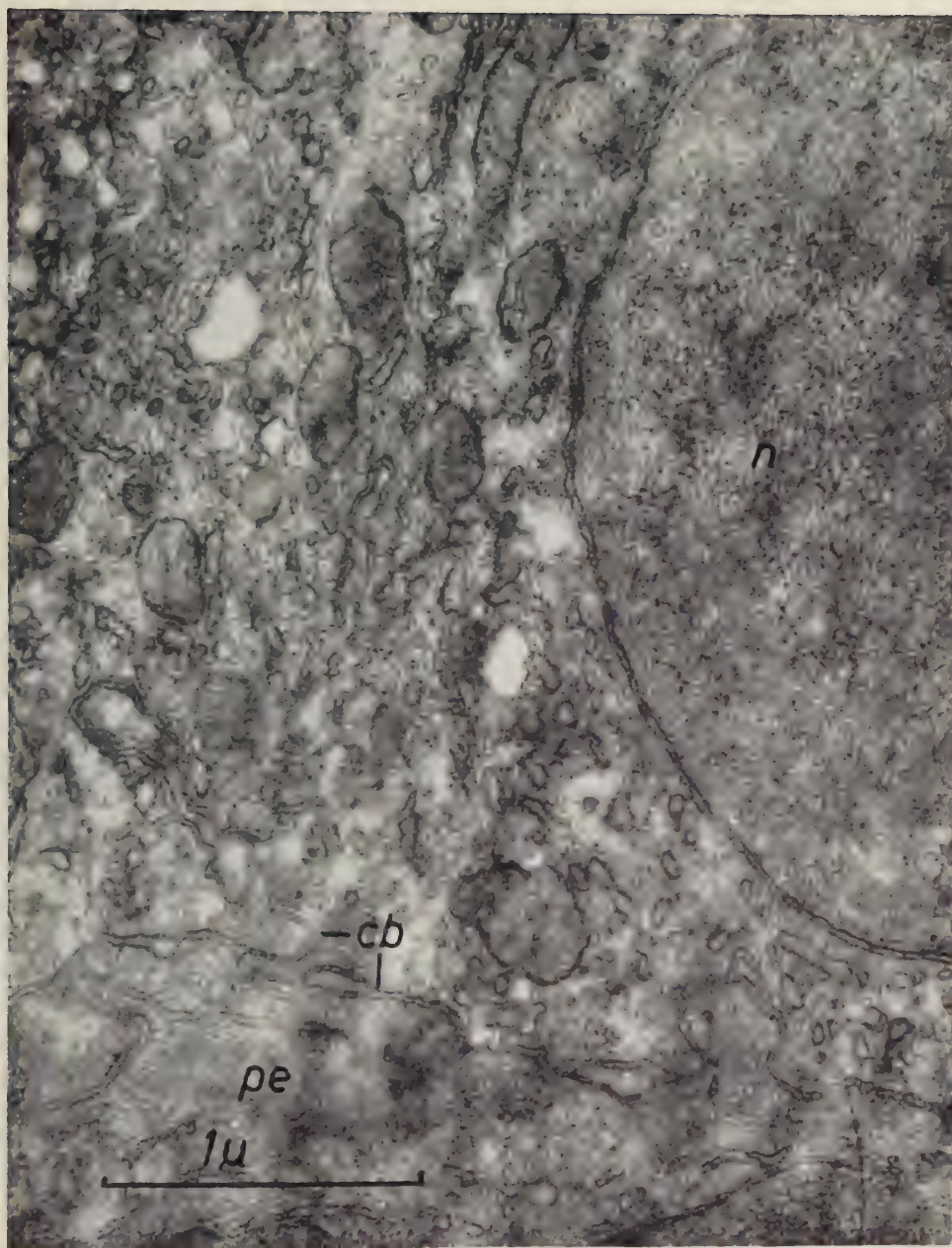


Fig. 39. Diamox 30 minutes. In the cytoplasm between the nucleus (n) and the cell border (cb) on the pigment epithelium the number of vesicles is much reduced as compared with Figs. 37 and 38. Magnification 67,000 $\times$.

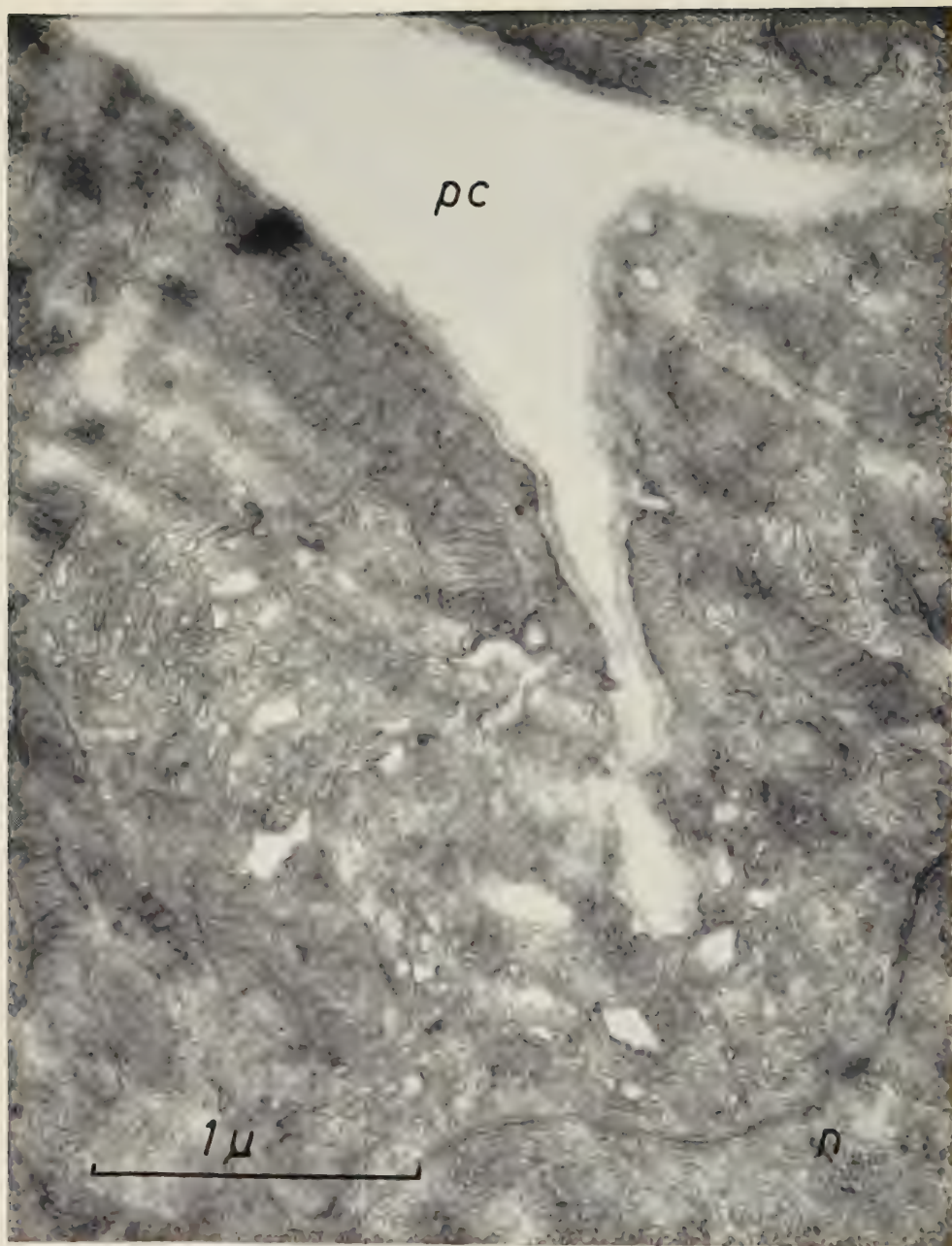


Fig. 40. Diamox 15 minutes. Apical part of a cell with the posterior chamber (pc). Above the nucleus (n) are seen many vesicles in the cytoplasm. The vesicles are arranged in rows. Magnification 43,000 x.

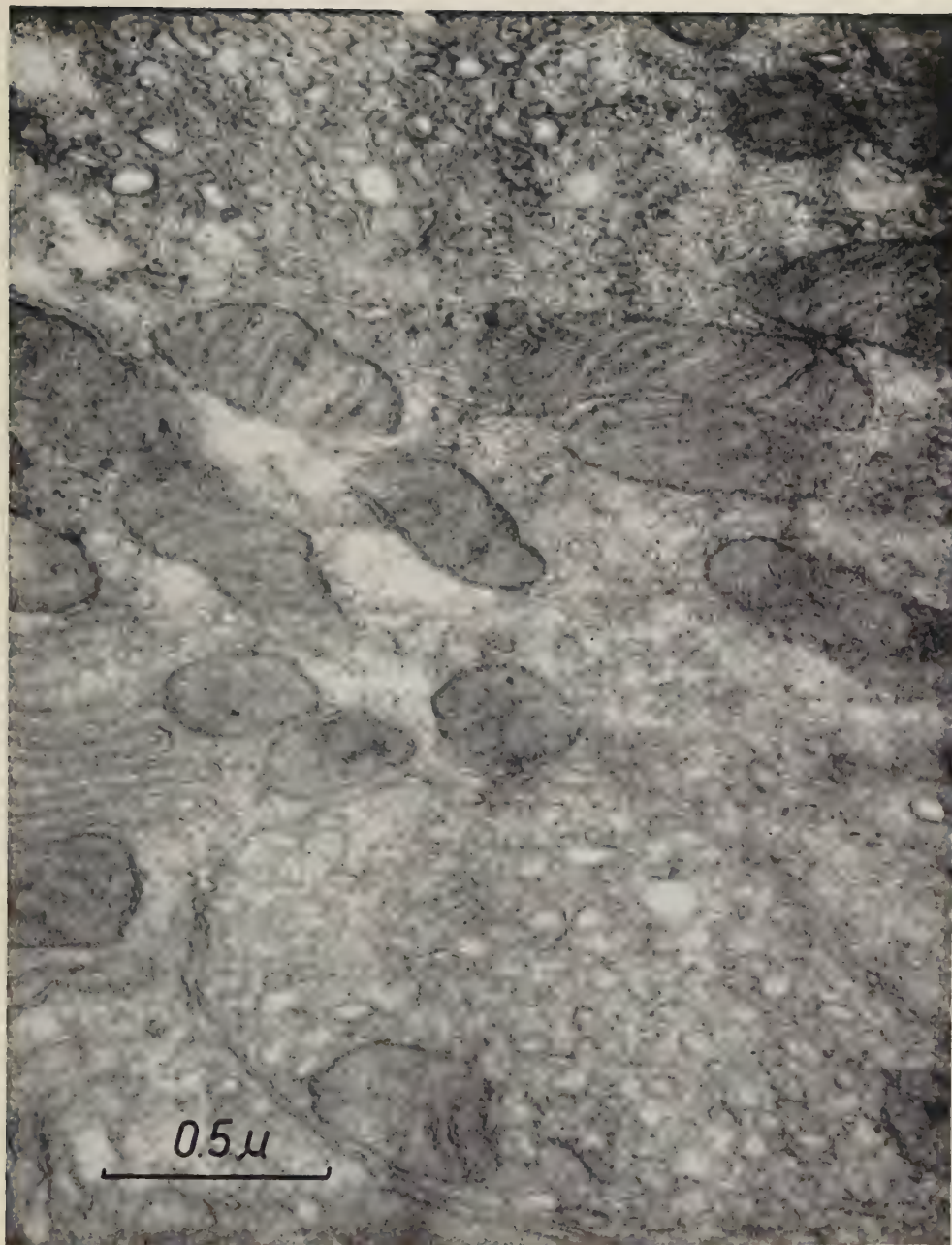


Fig. 41. *Diamox* 15 minutes. Groups of vesicles are seen. In the lower right of the picture part of an adjacent cell with its cell border is visible. Magnification 60,000 $\times$.



Fig. 42. *Diamox* 30 minutes. Apical part of a cell. In the upper corners the posterior chamber (pc) is visible. Above the nucleus (n) an assembly of vesicles lies. Parallel to a β -cytomembrane a long chain (arrows) of vesicles is seen. Magnification 51,000 x.

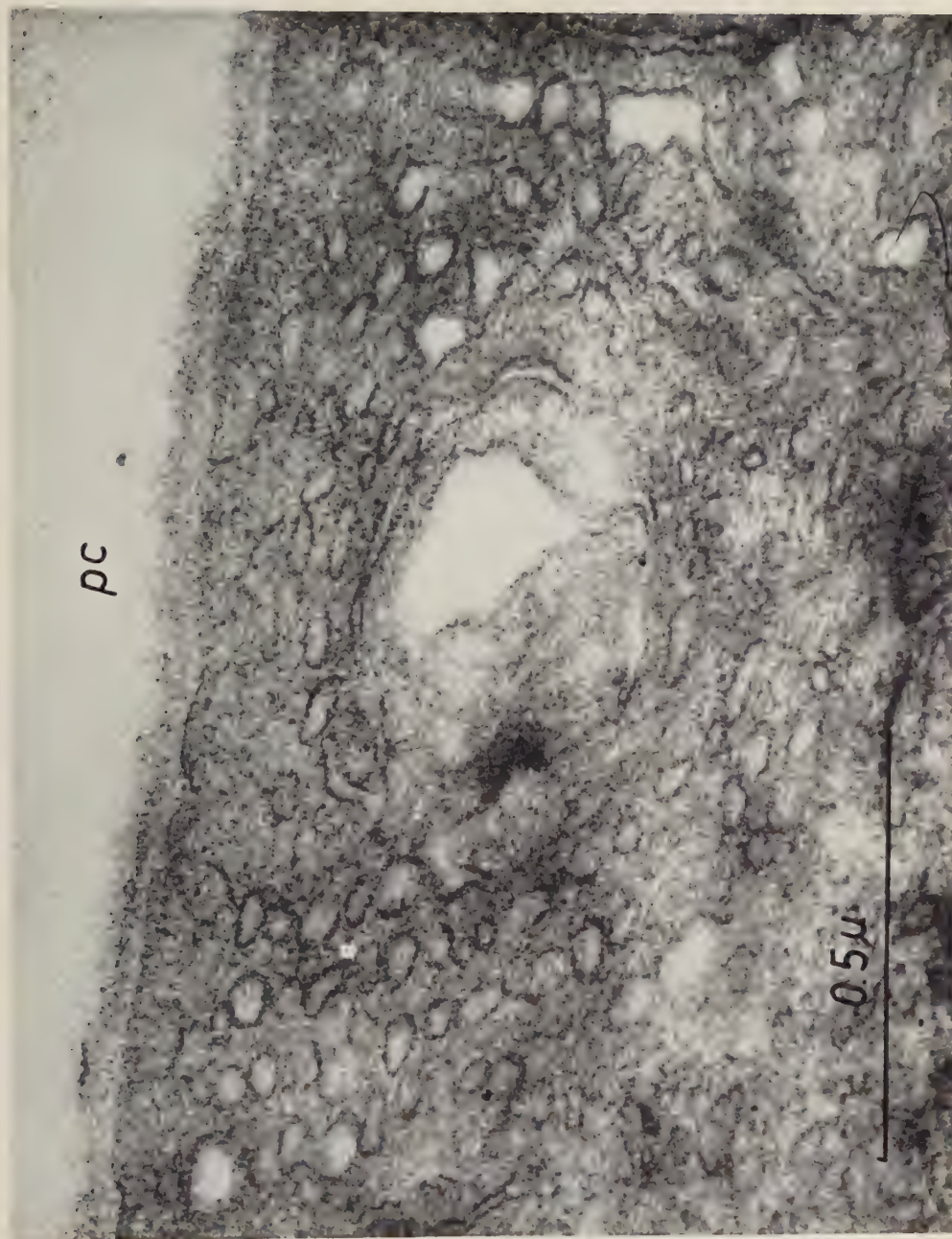


Fig. 43. Diamox 15 minutes. Detail of the apical part of a cell. The vesicles are arranged in rows or concentric arcs. Magnification 120,000 $\times$.



Fig. 44. *Diamor* 60 minutes. Close to the cell surface (pc = posterior chamber) the vesicles lie in long parallel rows. In some places short double membranes can be seen. Magnification 110,000 $\times$

It can clearly be seen from these curves how rapidly the change in frequency takes place with a sharp decrease in frequency from group 5 to group 6 and from group 6 to group 8. The curves, representing the Diazil-treated animals on the other hand however lie on a constant low level.

The analysis here referred to was only directed to the frequency of the vesicles in the different groups of animals and in different parts of the cells. A condition that is striking where the Diamox-treated animals are concerned is the arrangement of the vesicles in relation to each other. In Figs. 40—44 it is shown how next to the free surface of the cell in certain areas they lie arranged in long rows or concentric arcs. One gains the impression that the vesicles lie arranged in the same manner as the β -cytomembranes. This is shown clearly by Fig. 42 where, parallel to a β -cytomembrane, a long chain of vesicles lies beautifully arranged. Here and there one can see how the boundary membrane of a vesicle is in direct contact with the cell membrane (Fig. 43). Moreover, here and there in the rows of vesicles one can see short double membranes (Fig. 44).

This arrangement of the vesicles occurs, as stated earlier, in normal animals also (Fig. 7) but to a strikingly less extent. It would also appear as if an assembly of vesicles in this way occurs more often in the animals treated for a short time with Diamox (mainly groups 5 and 6). The difficulty in arriving at a sure basis for judgment and the comparatively small material have meant that no thorough analysis in this respect was carried out.

DISCUSSION

Lowering of the intra-ocular tension after injection of Diamox is now such a well known thing that any thorough discussion of this point does not seem to be required here. It will suffice to point out that all the Diamox-treated animals in this investigation showed a clear tension fall, whereas the Diazil-treated animals were not affected.

The study demonstrates that after injections of Diamox marked change in the ultrastructure of the non-pigmented ciliary epithelium takes place which appears to run parallel with the functional change. The clear correlation between the functional and morphological changes is impressive. As far as possible, measures were taken in this investigation to enable side effects of Diamox to be excluded. By working with nephrectomized animals the diuretic action of Diamox and the disturbances of fluid balance associated with that have been entirely eliminated. On such animals, moreover, the dosage of Diamox could be reduced to such a size that, while it had full effect on the intraocular

tension it could reasonably only slightly affect other body functions, such as respiration. Further, in the control material a solution (Diazil) was used very similar both chemically and physically with Diamox. Finally in intact animals with high doses of Diamox, exactly the same changes were observed as in the nephrectomized animals. As far as can be judged therefore the conclusion seems justified that the changes in the ultrastructure of the cells of the ciliary epithelium are related to the observed changes in the ocular tension. As stated in the introduction to this chapter, it is highly probable that Diamox has a specific limiting effect on the aqueous humour production by inhibition of the carbonic anhydrase. In view of the obvious correlation between the reduction in the tension of the eye and the changes in the ultrastructure of the cells, it seems reasonable to assume that the inhibition of the production of aqueous humour is caused by the changes in the cell structure.

Mitochondria

A marked change in the mitochondria both as regards number and as regards ultrastructure was demonstrated by Rhodin (1954) in the epithelium in proximal kidney tubules, after injection of egg white into mice. There was observed loss of ultrastructure and clumping of the mitochondria. These changes, though reversible, would seem rather to correspond to a pathological state than to a physiological variation, however. In the liver there has been observed (Schultz *et al.*, 1956) a great swelling of the mitochondria with clearing of the ground substance and increase of width of the outer and inner membranes in thyroxine-treated animals. It should also be stated here that pronounced changes in the mitochondria set in post mortem (Rhodin 1954, Zetterqvist 1956). The importance of knowing about this condition should be stressed, especially when it is a matter of judging changes produced experimentally.

The mitochondria of the ciliary epithelium after Diamox injection, develop a significant increase in width which, judging by the well retained ground substance, contrary to the above cited liver mitochondria, is caused by an increase in their ground substance. This increase takes place as early as 15 minutes after injection, whereas after this lapse of time no further change in mitochondria size appears to arise. Moreover, it is noteworthy that all the animals, irrespective of the time after the injection, reacted uniformly.

The change in the mitochondria appears to be more related to a changed functional state than solely to the time the change in the function sets in. As in the present case when the aqueous humour production and the cell

activity associated with it are diminished, one might reasonably regard the mitochondria change as an expression of a decrease in the energy which the mitochondria make available.

The Golgi apparatus

No certain change in the ultrastructure of the Golgi apparatus in conjunction with functional changes has previously been observed in vertebrates. A splitting of γ -cytomembrane piles has been observed, however in growing oocytes in sea urchins (Afzelius 1956).

Contrary to conditions in the mitochondria, the changes in the Golgi apparatus are to a large extent solely related to the time the tension fall takes place, while a certain return to normal conditions sets in when the tension is stabilised. Nevertheless, complete normalising of the number of vesicles or of the length of the membrane system do not occur in the times covered by this investigation. It is also striking that individual animals reacted very differently to Diamox from the morphological point of view, particularly as regards the amount of vesicles. It is possible that this variance in the changes in the Golgi apparatus is connected with how rapidly the tension fall occurs and how large it is. No thorough analysis has been performed in this respect.

The change in the amount of vesicles, the number of Golgi units and the change in the length of the membrane system proceeds in such a way that the number of vesicles and Golgi units are largest when the length of membrane system is least (Table 10, Textfigure 5). It appears natural to interpret this that the membrane system under the action of Diamox breaks up into several units and that in each unit the γ -cytomembranes break up into vesicles. Another explanation may be that the membrane system is built up of vesicles but that this process is prevented or arrested by Diamox. This latter explanation, however, appears less probable, taking into account that there arises a larger number of Golgi units than normal, especially after a short time of Diamox treatment.

The relation between the changes in the Golgi apparatus and the lowering of the intra-ocular tension seems evident. On the other hand, it is not possible to state the causal relationship between these two changes. If the first of the above-given interpretations were correct this would mean that the visible changes in the Golgi apparatus were a primary process, whereas according to the other interpretation the change would be secondary to another process not observable by the method used here. It seems likely, however, that the Golgi apparatus contributes in some way to the aqueous humour production.

The cytoplasm

The great increase in the frequency of vesicles in the cytoplasm lies in correlation to the initial stages of the Diamox action, but it should be noted that even after the intra-ocular tension has stabilised the frequency is still about 50 % higher compared with equivalent control animals. The changes in the cytoplasm are thus in this respect quite similar to those in the Golgi apparatus.

It may be assumed that during normal intra-ocular tension, a certain transfer of fluid takes place from the blood vessels through the cells of the ciliary epithelium to the posterior eye chamber. If the delivery of fluid from the cells to the posterior eye chamber suddenly decreases, which is assumed to be the case after Diamox injection (Becker, 1955, a, b), it is likely for there to be at first a considerable accumulation of fluid in the cells before the supply from the blood vessels is adapted to the reduced fluid delivery. It is possible that the fluid which is transferred through the cells is enclosed right from the beginning in vesicles in accordance with the opinion of Palade (1953 c), or it may be thought that the cells protect themselves against accumulation of fluid, by enclosing it in vesicles.

As stated earlier, there is no evidence that the vesicles are in movement towards the surface of the cell. No definite evidence could be obtained in this investigation that such is the case. The difference in frequency of vesicles in the different zones at different times after the Diamox injection varies so much in the different animal groups that no significant statistical basis was obtained that this difference is changed under the action of Diamox. As the rise in the frequency of the vesicles is most pronounced during the time the tension fall is taking place, it may be supposed that the variation in the animal groups is related to the time the tension fall starts, how rapidly the tension fall proceeds or how great it is.

From the functional point of view a similar result from Diamox administration, can be obtained if fluid passes from the posterior eye chamber to the cells. It is doubtful whether such a resorption is possible, in view of the fact that the aqueous humour has a certain amount of osmotic pressure, even after giving Diamox (Becker 1955 b). But even if a resorption were conceivable it is difficult to explain why the frequency of vesicles in animals where the tension is already stabilised, and where consequently a resorption from the functional point of view is unnecessary, is markedly greater compared with control animals. Moreover it is improbable that with a resorption one would get such a great fluid accumulation in the cells compared with normal conditions. One would have to assume that there is a barrier for the passage of the fluid from the cells to the blood path.

Even though, from the morphological viewpoint, the vesicles in the cytoplasm are identical with the vesicles in the Golgi apparatus there is no sure ground for supposing that there exists a relation between them. The change in the frequency of vesicles in the cytoplasm after Diamox injection proceeds much more quickly than the change in the quantity of vesicles in the Golgi apparatus. This does not support the idea that the vesicles in the cytoplasm come from the Golgi apparatus.

The strikingly regular arrangement, which the vesicles have at the cell surface here and there, would appear to occur more abundantly in the Diamox-treated animals than in the control animals. The striking similarity of arrangement between the vesicles and the β -cytomembranes appears strongly to indicate a certain relation between these two cell components. The explanations for finding more often after Diamox treatment such accumulations of vesicles regularly arranged in rows can be many. One explanation may be that the vesicles normally join up into membranes, but that under the action of Diamox this joining up is prevented or arrested. Another explanation may be that the β -cytomembranes under the action of Diamox break up into vesicles. In the former case the changes observed would be secondary to a process preceding them, in the latter they can be considered as primary. The relation between the vesicles and the β -cytomembranes and the changes in these after Diamox treatment would then be comparable to the relations in the Golgi apparatus between vesicles and membranes.

GENERAL DISCUSSION

In chapter II and III the ultrastructure of the non-pigmented epithelium has been thoroughly discussed both during normal conditions and after inhibition of the secretion of aqueous humour. In the present discussion some main problems will be touched on regarding the fixation for electron microscopy, as well as the function of the ciliary epithelium. Finally the mode of action of Diamox will be discussed.

The fixation

Osmium tetroxide has proved to be the most suitable fixative for electron microscopy. In this investigation phosphotungstic acid has been used in order to increase the contrast. This agent is generally used in the study of the ultrastructure of skeletal muscle, but it seems to be rarely used in connection with the studies of other types of cells. The experience from this investigation shows however, that phosphotungstic acid is rather generally suitable as a contrast enhancing electron stain. The fact, that the contrast in osmium-stained tissues is increased to a great extent by phosphotungstic acid is a further support of Sjöstrand's assumption (1953 a, d, f) that the opaque layers in the membrane (the double membranes of the mitochondria, the α -cytomembranes and so on) is a layer of protein.

An important problem in fixation is the artefact problem. The appearance of post mortem changes, for instance, has been given rather little attention. Rhodin (1954) and Zetterqvist (1956) have shown that great changes arise in the mitochondria *post mortem*, and Sjöstrand and Hanzon (1954) have shown that the α -cytomembranes and the mitochondria in the excretory cells of pancreas are deranged. It is extremely important to be aware of these circumstances, especially when judging about experimentally induced changes. Further examples of cell structures, which are changed *post mortem*, are the β -cytomembranes, the nuclear membrane, the nuclear substance and the ground substance of the cytoplasm. This has been mentioned in the discussion of the various structures, and it shall be pointed out, further, that some published interpretations of the cell components and their relationships would have been different if attention had been paid to the artefact problem. Owing to the method of fixation, which has been used in this investigation there have been as fortunate conditions as are possible to achieve for the present for

preventing post mortem changes. This seems anyhow justified to conclude from the fact that the tissue, has been fixed in the living state. During the course of this investigation it was possible to make direct comparisons between cells fixed *in vivo*, and cells fixed in the usual way (*post mortem*). The β -cytomembranes and the nuclear membrane of cells fixed *post mortem* showed a marked swelling of the osmiofobic layers as well, as large folds and interruptions in the osmiofilic layers.

The function of the ciliary epithelium

During the fourth decade of this century Duke-Elder's theory of the production of aqueous humour was generally accepted. According to this theory the aqueous humour was produced by a dialysis through membranes with a fixed permeability. The ciliary epithelium took no active part in the production. Several later investigations have shown however, that the production of aqueous humour was not such a simple process. The study of the rate of passage of different substances from the blood to posterior eye chamber showed that the rate to a certain extent was independent of the molecular weight. Urea, for instance, with molecular weight 60, has a rate of passage, which is only half as great as the rate of passage of glucose, with molecular weight 180 (Duke-Elder *et al.*, 1949). There are no substances, which are "freely diffusible" (Palm 1946). Therefore it is clear, that some kind of a selective barrier exist in the ciliary body. After experiments with acid and basic dyes and after measuring the potential between the stroma and the epithelium Friedenwald (1938) considered that the barrier lay in the stroma, but no morphological basis could be observed. Therefore Friedenwald assumed that the barrier consisted of a monomolecular layer. The rôle of the ciliary epithelium in the production of aqueous humour appeared clearly when Kinsey (1953) showed that the aqueous humour has a much higher content of bicarbonate than the blood. Contrary to Duke-Elder's opinion the ciliary epithelium is thus highly active in the production of aqueous humour and consequently Seidel's theory from 1918—1920 is actualized.

Contrary to Duke-Elder's opinion of the ciliary epithelium as a passive tissue in relation to aqueous humour production, the observations made in this investigation agree more with Friedenwald's idea that the ciliary epithelium participates actively in that production by a secretory process.

It would appear advisable to consider the function of the ciliary epithelium on the basis of the changes observed after inhibition of the aqueous humour production with Diamox. These changes may be summarised in the following points:

1. The mitochondria are enlarged.
2. The β -cytomembranes are split up.
3. The γ -cytomembranes are split up.
4. The vesicles in the cytoplasm increase considerably in number.

There proceeds in the mitochondria a coordinated enzymatic activity, through which energy is made available for the work of the cells. The fact that the mitochondria undergo a change in conjunction with a change in the aqueous humour production may indicate some effect on the energy generation in the cells, possibly in the form of diminished mitochondria activity. Direct studies of enzyme activity in isolated mitochondria fractions are required to arrive at conclusions in this respect. The increase in the width of the mitochondria may possibly be explained by an accumulation of substrate in the ground substance of the mitochondria.

As regards the other changes in the cell (splitting of the β - and γ -cytomembranes and increase of the vesicles in the cytoplasm) these may all be explained as a consequence of a general action of Diamox on the membrane system of the cell. It is probable that the membrane systems we are here concerned with are the seats of enzymatic activity. These active membranes would seem to collaborate in the transformation of the tissue fluid to aqueous humour. As regards the β -cytomembranes, it is also conceivable that they have the ability to regulate the delivery of fluid from the cells to the posterior chamber. Thus, if one produces a break down of these active structures it is understandable that there will be a decrease in aqueous humour formation. Against the assumption that Diamox would lead to a general break down of the membrane structures there is the circumstance that the cell membrane in the portion where it does not form β -cytomembranes is quite intact, as well as for instance the mitochondria membranes. Moreover that assumption is contradicted by the fact that the break down is only associated with the earlier stages of Diamox action, and does not remain throughout the action of Diamox.

If one assumes the vesicles as transport bodies for the aqueous humour the great accumulation of them in the cytoplasm may be explained in such a way that Diamox produces an inhibition of the transport mechanism. Functionally, that should naturally lead to a diminished formation of aqueous humour. Nevertheless, there is no evidence that the vesicles really do contain aqueous humour. Moreover, it is difficult with this manner of thinking to link together the changes in the Golgi apparatus and the β -cytomembranes.

One explanation of all the cell changes after administration of Diamox, except the changes of the mitochondria, may be obtained on the basis of the

β - and γ -cytomembranes. The β -cytomembranes may, as stated above, be assumed to constitute a barrier which in the last stage regulates the aqueous humour production by enzymatic activity. The γ -cytomembranes on the other hand may be assumed to produce enzymatically active membrane fragments in the form of vesicles which are carried to the cell surface, where they join together to form β -cytomembranes. If now Diamox inhibits the activity of the β -cytomembranes, which functionally leads to a diminished aqueous humour formation, this inhibition may be compensated by an increased production of new active membranes in the Golgi apparatus. The changes in the Golgi apparatus in the earlier stages of Diamox action would also appear more to give the impression of increased activity.

The changes in the Golgi apparatus after inhibition of the enzyme activity in the β -cytomembranes, with partial blockage of the delivery of aqueous humour according to the assumption above, may also be interpreted in such a way that there takes place an increased formation of vesicles in the Golgi apparatus to guard the cells against an excessive accumulation of the aqueous humour which is formed but cannot be delivered. The vesicles may, in fact, have the mission of separating in high concentrations the ions which are concentrated by the secretion process, so that swelling of the cytoplasm with a disturbed composition of the cytoplasm is prevented.

The two last interpretations may thus together provide an explanation of the changes in the β - and γ -cytomembranes and the cytoplasm. It was not possible, however, to demonstrate in this investigation that the vesicles in the cytoplasm are in movement towards the cell surface. Nor could any definite relations between the vesicles in the Golgi region and the vesicles in the cytoplasm be demonstrated, apart from the purely morphological resemblance.

The mode of action of Diamox

It is, of course, not possible in this investigation to decide in what manner Diamox acts chemically on the aqueous humour production. Nevertheless, it appears evident that Diamox has a local action on the ciliary epithelium. From the discussion presented above it appears that Diamox may have many points of attack. It may act on the enzyme activity in the mitochondria, or it may act on the activity of the β - and γ -cytomembranes. The most probable assumption is that Diamox produces a blockage in the β -cytomembranes.

SUMMARY

The analysis of the non-pigmented epithelial cells of the ciliary body was done on osmium-fixed tissues. A concentration of 1 % osmium tetroxide in a veronal acetate buffered, blood isotonic salt solution was used. The fixing procedure was started *in vivo* with the animals under nembutal narcosis. During dehydration 0,1 % phosphotungstic acid in 70 % alcohol was used in order to increase the contrast in the tissue. The specimens were embedded in methacrylate and were sectioned on a Sjöstrand-Ultramicrotome. The sections were studied in RCA EMU 2 c electron microscope.

The analysis of the β -cytomembranes in the ciliary epithelium shows that they consist of irregular folds of the cell membrane. They occur only in the apical parts of the cell. They appear as double membranes about 160 Å thick, with two opaque layers bordering a light central layer. No communication was observed between the β -cytomembranes, the α -cytomembranes and the γ -cytomembranes.

The α -cytomembranes are in most cases located in groups in limited regions in the apical part of the cells. They occur as double membranes, forming closed areas. The thickness of each membrane is about 40 Å and on one side osmiophilic particles are attached, about 150 Å in diameter. The distance between the membranes in a pair varies between 180 and 760 Å.

The Golgi apparatus is located in the basal parts of the cell. In each cell there occur 1—2 Golgi units, composed of a number of membranes, some large vacuoles, several vesicles and possibly a ground substance. The membranes are paired and arranged in 3—6 parallel rows. The thickness of each membrane is about 50 Å and the distance between membranes from neighboring pairs is about 60 Å. Through widening of the space between the membranes in a membrane pair large vacuoles are formed. The vesicles have a very intimate relationship to the membranes. They measure between 250 and 800 Å in diameter, and are bordered by a membrane about 50 Å thick.

At the border between two adjacent cells, where two cell membranes are in close contact, a double membrane is formed, about 160 Å thick. This membrane is very folded in the apical regions. In the cytoplasm extending into the folds there lies a thin opaque layer. From the thickness of the double membrane the thickness of the cell membrane was calculated to 80 Å with an opaque component about 40 Å.

Along the border on the pigment epithelium there was encountered a large number of *terminal bars*. In the region of the terminal bar the opaque component of the cell membrane is split up into three layers, each about 20 Å thick. The central light layer in the cell border is widened to about 140 Å within the terminal bars.

Outside the free surface of the cell there is a *basement membrane*, about 300 Å thick. The distance between this and the cell membrane varies between 400 and 700 Å. Thin bands seem to connect the basement membrane and the cell membrane.

The *mitochondria* have a width of about $0.18\ \mu$. The outer double membrane is about 130 Å thick and the inner double membranes are about 150 Å thick. The opaque layers in the membranes are about 40 Å thick. The inner membranes are in most cases oriented perpendicular to the outer membrane. In some mitochondria, however, one to five inner membranes were arranged parallel with the long axis of the mitochondria. In these mitochondria the inner membranes are about 200 Å thick, and each opaque layer is about 60 Å thick.

The *nucleus* is surrounded by a double membrane, about 190 Å thick. The inner opaque layer is about 70 Å thick, the central light layer is about 75 Å thick and the outer opaque layer is about 45 Å thick. There are no folds in the opaque layers and the central layer is very uniform. "Holes" very few in number, occur in the nuclear membrane. The "holes", however, are bridged by a thin membrane. The nuclear substance consists of opaque particles, about 150 Å in diameter. They are uniformly distributed throughout the nucleus. The nucleolus is made up of closely packed particles.

Two types of *large granules* occur in the ciliary epithelium. One of them is bordered by a double membrane, about 60 Å thick. This granule is filled either with a structureless substance or with small vesicles, 400—500 Å in diameter. The other granule is most often encountered in the basal parts of the cell. It is bordered by a single membrane, about 40 Å thick. It is filled with 40—70 Å grains which are strongly osmophilic.

In the ciliary epithelium a cell component was encountered, which is assumed to be a separate unit. It is here called *large spherical body*. It occurs only in the apical part of the cell. The smallest diameter is between 0.23 and $0.48\ \mu$ and the largest diameter is between 0.47 and $1.0\ \mu$. It is surrounded by a double membrane, about 150 Å thick. It is built up of four components: a solitary granule 900—1,200 Å in diameter, several small particles 350—450 Å in diameter, a number of 150 Å particles and a ground substance, which is less dense than that of the cytoplasm.

In the ground substance of the cytoplasm there occur *particles* about 150 Å in diameter, *vesicles* 300—1.000 Å in diameter, surrounded by a membrane about 40 Å thick, and a homogeneous matrix, which appears structureless.

The effect of Diamox. Fourteen nephrectomized animals and three not nephrectomized animals have been treated during varying periods with Diamox, intravenously administrated. A lowering of the intraocular tension between 7 and 20 mm Schiötz has been obtained in all these animals. In ten control animals, treated with Diazil instead of Diamox, no lowering of the intraocular tension was registered.

After injection of Diamox marked changes of the ultrastructure of the ciliary epithelium were observed, while no changes were observed in the Diazil-treated animals. The mitochondria show a significant increase in their width from 0.18 μ to 0.25 μ . The change sets in already 15 minutes after the injection and from this time on there is no further change. Only in those mitochondria, which showed an extreme increase in width changes in the membrane systems were observed.

In the Golgi apparatus there is a great increase of the number of vesicles with maximum 15—60 minutes after the injection. After 120 minutes the number of vesicles is significantly reduced, but not to the level of the control animals. Concurrently with the increase in the number of vesicles, there is a reduction of the length of the rows of the membrane pairs. In the control animals the average length is about 0.60 μ . Fifteen to thirty minutes after injection of Diamox, the length is about 0.40 μ . After longer periods of Diamox treatment the length increases again but not to that of the control animals.

In the cytoplasm there is observed an enormous increase in the frequency of vesicles with maximum 15 minutes after the injection of Diamox. At this time the frequency is four to five times greater than that in the control animals. After longer periods of Diamox treatment the frequency is rapidly decreased, but 120 minutes after the injection it is still 50 % greater than that of the control animals.

Close to the cell surface the vesicles become arranged in rows or concentric arcs, strikingly similar to the arrangement of the β -cytomembranes.

The changes in the ultrastructure have been correlated to the inhibition of the production of aqueous humour. Several ways have been discussed, in which the different cell constituents may take part in the production and produce the inhibition of the production.

REFERENCES

- Adler, F. H.: Is the aqueous humor a dialysate. *Trans. Am. Ophthalmol. Soc.* 31, 131, 1933.
- Afzelius, B. A.: The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope. *Exptl. Cell Research.* 8, 147, 1955. a.
- Afzelius, B. A.: The fine structure of the sea urchin spermatozoon as revealed by the electron microscope. *Z. Zellforsch. u. mikroskop. Anat.* 42, 134, 1955 b.
- Afzelius, B. A.: Electron microscopy of Golgi elements in sea urchin eggs. *Exptl. Cell Research.* 11, 67, 1956.
- Albrich, K.: Ueber die sekretion des Ziliarkörperepithels. *Klin. Monatsbl. Augenheilk.* 71, 781, 1923.
- André, J.: L'ultrastructure des ovocytes d'araignée. *Proc. First Europ. Reg. Conf. Electron Microscopy, Stockholm 1956.* In press.
- Bairati, A. und Lehmann, F. E.: Ueber die submikroskopische Struktur der Kernmembran bei *Amoeba proteus*. *Experientia.* 8, 60, 1952.
- Bárány, E.: The mode of entrance of sodium into the aqueous humour. *Acta Physiol. Scand.* 13, 55, 1947 a.
- Bárány, E.: The relative importance of ultrafiltration and secretion in the formation of aqueous humour as revealed by the influence of arterial blood pressure on the osmotic pressure of the aqueous. *Acta Physiol. Scand.* 13, 81, 1947 b.
- Bárány, E.: The action of Atropine, Homatropine, Eserine and Prostigmine on the osmotic pressure of the aqueous humour. *Acta Physiol. Scand.* 13, 95, 1947 c.
- Bárány, E.: The influence of Gum arabic and Dextran on the blood-aqueous barrier and introcular pressure. *Ophthalmologica.* 116, 65, 1948.
- Bargmann, W., Knoop, A. und Schiebeler, Th. H.: Histologische, cytochemische und elektronenmikroskopische Untersuchungen am Nephron. *Z. Zellforsch. u. mikroskop. Anat.* 42, 386, 1955.
- Beams, H. W., Tahmisian, T. N. and Devine, R. L.: Electron microscope studies on the cells of the malpighian tubules of the grasshopper. *J. Biophys. Biochem. Cytol.* 1, 197, 1955.
- Becker, B.: Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox. *Am. J. Ophthalmol.* 37, 13, 1954 a.
- Becker, B.: The short-term administration of Diamox in the therapy of the glaucomas. *Acta XVII Conc. Ophthalmol.* 1954 b.
- Becker, B.: The mechanism of the fall in intraocular pressure induced by the carbonic anhydrase inhibitor, Diamox. *Am. J. Ophthalmol.* 39, 177, 1955 a.

- Becker, B.: The effects of the carbonic anhydrase inhibitor, acetazoleamide, on the composition of the aqueous humor. *Am. J. Ophthalmol.* 40, 129, 1955 b.
- Becker, B.: The present status of the clinical use of Diamox in the management of the glaucomas. *Southern Med. J.* 48, 866, 1955 c.
- Becker, B. and Middleton, W.: Long-term acetazoleamide (Diamox) administration in therapy of glaucomas. *Arch. Ophthalmol.* 54, 187, 1955 d.
- Becker, B. and Constant, M.: The alterations in aqueous humor lactate concentration, following systemic carbonic anhydrase inhibition. *Am. J. Ophthalmol.* 42, 406, 1956.
- Bernhard, W., Haguenau, F. et Oberling, Ch.: L'ultrastructure du nucléole de quelques cellules animales, révélée par le microscope électronique. *Experientia.* 8, 58, 1952.
- Bernhard, W., Haguenau, F., Gautier, A. et Oberling, Ch.: La structure submicroscopique des éléments basophiles cytoplasmique dans le foie, le pancréas et les glandes salivaires. *Z. Zellforsch. u. mikroskop. Ant.* 37, 281, 1952.
- Binder, R. und Orth, E.: Elektronenoptische Studie an Pigmentkörnern und Zonulafasern des menschlichen Auges. *Graefes Arch. Augenheilk.* 154, 266, 1953.
- Birch-Andersen, A. and Maaløe, O.: High-resolution electron micrographs of sections of *E. Coli*. *Biochem. Biophys. Acta.* 12, 395, 1953.
- Burgos, M. H. and Fawcett, D. W.: Studies on the fine structure of the mammalian testis. *J. Biophys. Biochem. Cytol.* 1, 287, 1955.
- Callan, H. G. and Tomlin, S. G.: Experimental studies on amphibian oocyte nuclei. 1. Investigation on the structure of the nuclear membrane by means of the electron microscope. *Proc. Roy. Soc. (London)*, B, 137, 367, 1950.
- Carlini, V.: Die Veränderungen des Iris und Ziliarepithels nach Punktion der Vorderkammer; Beitrag zum Studium des Produktionsmechanismus des Humor aqueus. *Arch. Ophthalmol.* 77, 96, 1910.
- Carrère, L.: Histologie de la région ciliaire de la rétine chez le lapin albinos. *Compt. rend. Mem. Soc. Biol.* 88, 420, 1923.
- Clermont, Y.: The Golgi zone of the rat spermatid and its role in the formation of cytoplasmic vesicles. *J. Biophys. Biochem. Cytol. Suppl.* 2, 119, 1956.
- Czermak, W.: Zur Zonulafrage. *Graefes Arch. Augenheilk.* 31, 79, 1885.
- Dalton, A. J.: Electron micrography of epithelial cells of the gastrointestinal tract and pancreas. *Am. J. Anat.* 89, 109, 1951.
- Dalton, A. J.: A study of the Golgi material of hepatic and intestinal epithelial cells with the electron microscope. *Z. Zellforsch. u. mikroskop. Anat.* 36, 522, 1952.
- Dalton, A. J. and Felix, M.: Studies on the Golgi substance of the epithelial cells of the epididymis and duodenum of the mouse. *Am. J. Anat.* 92, 277, 1953.
- Dalton, A. J. and Felix, M.: Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis. *Am. J. Anat.* 94, 171, 1954.

- Dalton, A. J. and Felix, M.: A comparative study of the Golgi complex. *J. Biophys. Biochem. Cytol. Suppl.* 2, 79, 1956.
- Dalton, A. J., Kahler, H. and Lloyd, B.: The structure of the free surface of a series of epithelial cell types in the mouse as revealed by the electron microscope. *Anat. Record.* 111, 1, 1951.
- Dalton, A. J., Kahler, H., Lloyd, B. and Striebich, M. J.: Finer structure of the hepatic, intestinal and renal cells of the mouse as revealed by the electron microscope. *J. Nat. Cancer Inst.* 11, 439, 1950.
- Davson, H. and Danielli, J. F.: The permeability of natural membranes. Cambridge 1952.
- Davson, H., Duke-Elder, W. S., Maurice, D. M., Ross, E. J. and Woodin, A. M.: The penetration of some electrolytes and non-electrolytes into the aqueous humour and vitreous body of the cat. *J. Physiol.* 108, 203, 1949.
- Dawson, I. M., Hossack, J. and Wyburn, G. M.: Observations on the Nissl's substance, cytoplasmic filaments and the nuclear membrane of spinal ganglion cells. *Proc. Roy. Soc. (London).* B 144, 132, 1955.
- Dempsey, E. W. and Wislocki, G. B.: An electron microscopic study of the blood-brain barrier in the rat, employing silver nitrate as a vital stain. *J. Biophys. Biochem. Cytol.* 1, 245, 1955.
- Duke-Elder, W. S., Davson, H. and Woodin, A. M.: Studies on the intraocular fluids. *Brit. J. Ophthalmol.* 33, 452, 1949.
- Duke-Elder, P. M. and Duke-Elder, W. S.: Studies on the intraocular pressure. *J. Physiol.* 71, 268, 1931.
- von Ebner, V.: in Kölliker: Handbuch der Gewebelehre des Menschen. Leipzig 1902.
- Engström, H. and Sjöstrand, F. S.: The structure and innervation of the cochlear hair cells. *Acta Oto-Laryngol.* 44, 490, 1954.
- Falbriard, A., Zender, R., Sanz, M. C. and Franceschetti, A.: Le potassium et le sodium de l'humeur aqueuse du lapin et leurs variations sous l'effet de l'acétazolamide. (Diamox). *Experientia.* 11, 232, 1955.
- Fawcett, D. W.: Observations on the cytology and electron microscopy of hepatic cells. *J. Nat. Cancer Inst. Suppl.* 15, 1475, 1955.
- Finean, J. B., Sjöstrand, F. S. and Steinman, E.: Submicroscopic organisation of some layered lipoprotein structures. (Nerve myelin, retinal rods, and chloroplasts). *Exptl. Cell Research.* 5, 557, 1953.
- Friedenwald, J. S.: Dynamic factors in the formation and re-absorption of aqueous humour. *Brit. J. Ophthalmol.* 28, 503, 1944.
- Friedenwald, J. S.: The formation of the intraocular fluid. *Am. J. Ophthalmol.* 32, 9, 1949.
- Friedenwald, J. S.: Carbonic anhydrase inhibition and aqueous flow. *Am. J. Ophthalmol.* 39, 59, 1955.
- Friedenwald, J. S. and Becker, B.: Aqueous humor dynamics. *Am. J. Ophthalmol.* 41, 383, 1956.

- Friedenwald, J. S. and Pierce, H. F.*: The respiratory function of the aqueous. *Trans. Am. Ophthalmol. Soc.* 31, 143, 1933.
- Friedenwald, J. S. and Stiehler, R. D.*: Circulation of the aqueous. VII. A mechanism of secretion of the intraocular fluid. *Arch. Ophthalmol.* 20, 761, 1938.
- Friedenwald, J. S., Herrman, H. and Moses, R.*: The distribution of certain oxidative enzymes in the ciliary body. *Bull. Johns Hopkins Hosp.* 73, 421, 1943.
- Galdston, M., Subit, I., Hollis, V. W. Jr and Homer, Mary*: Respiratory and renal effects of a carbonic anhydrase inhibitor (Diamox) on acid-base balance in normal man and in patients with respiratory acidosis. *Am. J. Med.* XIX, 516, 1955.
- Gilbert, W.*: Ueber Veränderungen des Ziliarepithels nach Vorderkammerpunktion nebst Bemerkungen ueber Kammerwasserersatz. *Arch. Augenheilk.* 88, 210, 1921.
- Grant, W. M.*: Tonographic method for measuring the facility and rate of aqueous flow in human eyes. *Arch. Ophthalmol.* 44, 204, 1950.
- Hagen, S.*: Zur Arbeit von Wessely: Bemerkungen zu einigen Streitfragen aus der Lehre vom intraokularen Flüssigkeitswechsel. *Klin. Monatsbl. Augenheilk.* 66, 259, 1921.
- Hagen, S.*: Weitere Untersuchungen ueber die Regeneration des Kammerwassers im menschlichen Auge. *Klin. Monatsbl. Augenheilk.* 66, 493, 1921.
- Hall, C. E., Jakus, Marie and Schmitt, F. O.*: The structure of certain muscle fibrils as revealed by the use of electron stains. *J. Appl. Phys.* 16, 459, 1945.
- Harris, J.E. and Gehrsitz, L. B.*: The movement of monosacharides into and out of the aqueous humor. *Am. J. Ophthalmol.* 32, 167, 1949.
- Hartmann, J. F.*: An electron optical study of sections of central nervous system. *J. Comp. Neurol.* 99, 201, 1953.
- Hartmann, J. F.*: Electron microscopy of motor nerve cells following section of axones. *Anat. Record.* 118, 19, 1954.
- Hibbard, H.*: Current status of our knowledge of the Golgi apparatus in the animal cell. *Quart. Rev. Biol.* 20, 1, 1945.
- Hofmann-Credner, A., Locker, A. and Moser, K.*: Die Wirkung von Diamox auf die Gewebsatmung. *Z. ges. Exptl. Med.* 126, 546, 1955.
- Holmberg, Å.*: Studies of the ultrastructure of the non-pigmented epithelium in the ciliary body. *Acta Ophthalmol.* 33, 377, 1955.
- Kinsey, V. E.*: A unified concept of aqueous humor dynamics and the maintenance of intraocular pressure: An elaboration of the secretion — diffusion theory. *Arch. Ophthalmol.* 44, 215, 1950.
- Kinsey, V. E.*: Comparative chemistry of aqueous humor in posterior and anterior chamber of rabbit eye. *Arch. Ophthalmol.* 50, 401, 1953.
- Kinsey, V. E. and Bárány, E.*: Rate of flow of aqueous humor: derivation of rate of flow and its physiologic significance. *Am. J. Ophthalmol.* 32, 189, 1949.
- Kinsey, V. E., Palm, E. and Cavanaugh, G. A.*: Posterior and anterior chamber aqueous humor formation. *Arch. Ophthalmol.* 53, 330, 1955.

- Kirkman, H. and Severinghaus, A. E.: A review of the Golgi apparatus. *Anat. Record.* 70, 413, 1938.
- Kronfeld, P. C.: The regeneration of the aqueous humor. *Arch. Ophthalmol.* 1, 231, 1929.
- Lacy, D.: Chemical composition of the Golgi apparatus in the exocrine and endocrine cells in the pancreas of the mouse. *Nature.* 173, 1235, 1955.
- Larsson, S.: Ueber den Augendruck und die Vorderen intraokularen Gefäße. Stockholm 1930.
- Lauber, H.: Die mikroskopische Anatomie des Ziliarkörpers, der Aderhaut und der Glaskörpers. Graefe-Saemisch Handbuch der Augenheilk. 1:2, 1931.
- Leber, Th.: Die Augenflüssigkeiten, ihre Absonderung und ihr Abfluss. Graefe-Saemisch Handbuch der Augenheilk. 2:2, 207, 1903.
- Leplat, G.: E'tude de quelques réactions provoquées dans les deux yeux par une contusion oculaire unilatérale. Recherches de psysilogie et d'anatomo — pathologie. *Ann. Ocul.* 162, 82, 1925.
- Magitot, A.: L'humeur aqueuse et son origine. *Ann. Ocul.* 154, 65, 211, 1917.
- Mawas, J.: E'tudes cytologique et physiologique sur le rétine ciliaire des mammifères. *Arch. Anat. Microscopique.* 12, 103, 1910.
- von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen. 3:2, 176, 1936.
- Odor, L.: Observations of the rat mesothelium with the electron and phase microscopes. *Am. J. Anat.* 95, 433, 1954.
- Ottoson, D., Sjöstrand, F. S., Stenström, S. and Svaetichin, G.: Microelectrode studies on the E. M. F. of the frog skin related to electron microscopy of the dermo-epidermal junction. *Acta Physiol. Scand.* 29, 611, 1953.
- Palade, G. E.: A study of fixation for electron microscopy. *J. Exptl. Med.* 95, 285, 1952 a.
- Palade, G. E.: The fine structure of mitochondria. *Anat. Record.* 114, 427, 1952 b.
- Palade, G. E.: An electron microscope study of the mitochondrial structure. *J. Histochem. Cytochem.* 1, 188, 1953 a.
- Palade, G. E.: A small particulate component of the cytoplasm. *J. Appl. Phys.* 24, 1419, 1953 b.
- Palade, G. E.: Fine structure of blood capillaries. *J. Appl. Phys.* 24, 1924, 1953 c.
- Palade, G. E.: A small particulate component of the cytoplasm. *J. Biophys. Biochem. Cytol.* 1, 59, 1955.
- Palade, G. E. and Porter, K. R.: Studies on the endoplasmic reticulum. *J. Exptl. Med.* 100, 641, 1954.
- Palay, S. L. and Palade, G. E.: Fine structure of neuronal cytoplasm. *J. Appl. Phys.* 24, 1419, 1953.
- Palay, S. L. and Palade, G. E.: The fine structure of neurons. *J. Biophys. Biochem. Cytol.* 1, 69, 1955.

- Palm, E.*: On the penetration of various substance from the blood to central nervous system and the eye. *Acta Ophthalmol.* 24, 189, 1946.
- Palm, E.*: On occurence in retina of conditions corresponding to "blood-brain barrier". *Acta Ophthalmol.* 25, 29, 1947.
- Palm, E.*: Kammarvattnet och ögontryckregulationen. *Nord. Med.* 56, 1405, 1956.
- Pease, D.*: Electron microscopy of the vascular bed of the kidney cortex. *Anat. Record.* 121, 701, 1955 a.
- Pease, D.*: Fine structures of the kidney seen by electron microscopy. *J. Histochem. Cytochem.* 3, 295, 1955 b.
- Pease, D.*: Infolded basal plasma membrane found in epithelia noted for their water transport. *J. Biophys. Biochem. Cytol. Suppl.* 2, 203, 1956.
- Porter, K. R.*: Observations on a submicroscopic basophilic component of cytoplasm. *J. Exptl. Med.* 97, 727, 1953.
- Porter, K. R.*: Electron microscopy of basophilic components of cytoplasm. *J. Histochem. Cytochem.* 2, 346, 1954.
- Porter, K. R. and Kallman, F. L.*: Significance of cell particulates as seen by electron microscopy. *Ann. N. Y. Acad. Sci.* 54, 882, 1952.
- Porter, K. R. and Palade, G. E.*: The endoplasmic reticulum of cells in situ. *Anat. Record.* 112, 370, 1952.
- Porter, K. R. and Thompson, H. P.*: Some morphological features of cultured rat sarcoma cells as revealed by the electron microscope. *Cancer Research.* 7, 431, 1947.
- Rhodin, J.*: Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. Stockholm 1954.
- Rhodin, J.*: Electron microscopy of the glomerular capillary wall. *Exptl. Cell Research.* 8, 572, 1955.
- Rhodin, J. and Dalhamn, T.*: Electron microscopy of collagen and elastin in lamina propria of the tracheal mucosa of rat. *Exptl. Cell Research.* 9, 372, 1955.
- Rhodin, J. and Dalhamn, T.*: Electron microscopy of the tracheal ciliated mucosa in rat. *Z. Zellforsch. u. mikroskop. Anat.* 44, 345, 1956.
- Rintelen, F. und Jenny, E.*: Ueber "Diamox" therapie beim Glaukom. *Ophthalmologica.* 130, 171, 1955.
- de Robertis, E.*: The nucleo-cytoplasmic relationship and the basophilic substance (ergostoplasm) of nerve cells. (Electron microscope observations). *J. Histochem. Cytochem.* 2, 341, 1954.
- Robertsson, J. D.*: The ultrastructure of adult vertebrate peripheral myelinated nerve fibers in relation to myelinogenesis. *J. Biophys. Biochem. Cytol.* 1, 271, 1955.
- de Roethh, A. Jr.*: Respiration of the ciliary processes. *Arch. Ophthalmol.* 50, 491, 1953.
- Ross, E. J.*: The transfer of non-electrolytes across the blood-aqueous barrier. *J. Physiol.* 112, 229, 1951.

- Saltzmann, M.*: Anatomie und Histologie des menschlichen Augapfels im Normalzustande, seine Entwicklung und sein Altern. Leipzig. 1912.
- Schultz, H.*: Elektronenoptische Untersuchungen der normalen Lunge und der Lunge bei Mitralstenose. *Virchow's Arch.* 328, 582, 1956.
- Schultz, H., Löw, H., Ernster, L. and Sjöstrand, F. S.*: Electron microscopic studies of liver sections of thyroxinetreated rats. *Proc. First Europ. Reg. Conf. Electron Microscopy. Stockholm 1956.* In press.
- Seidel, E.*: Zur Physiologie des intraokularen Flüssigkeitswechsels. *Z. Augenheilk.* 40, 81, 1918.
- Seidel, E.*: Weitere experimentelle Untersuchungen ueber die Quelle und den Verlauf der intraokularen Saftströmung. *Arch. Ophthalmol.* 102, 189, 1920 a.
- Seidel, E.*: Ueber den Vorgang der physiologischen Kammerwasserabsonderung und seine pharmakologische Beeinflussung. *Arch. Ophthalmol.* 102, 366, 1920 b.
- Seidel, E.*: Ueber den Kammerwasserersatz im menschlichen und im Tier-Auge. *Arch. Ophthalmol.* 104, 162, 1921 a.
- Seidel, E.*: Ueber die physiko-chemischen Vorgänge im Ziliarepithel. *Arch. Ophthalmol.* 104, 284, 1921 b.
- Seidel, E.*: Ueber die Gewebesatmung im Auge und ihre klinische Bedeutung. *Klin. Monatsbl. Augenheilk.* 75, 194, 1925.
- Seidel, E.*: Methoden zur Untersuchung des intraokularen Flüssigkeitswechsels. *Handbuch der Biologischen Arbeitsmethoden.* 6:2, 1019, 1937.
- Serr, H.*: Ueber den Vorgang der Kammerwasserbildung. *Klin. Monatsbl. Augenheilk.* 81, 350, 1928.
- Sheldon, H. and Zetterqvist, H.*: An electron microscope study of the corneal epithelium in the vitamin A deficient mouse. *Bull. Johns Hopkins Hosp.* 98, 372, 1956.
- Sheldon, H., Zetterqvist, H. and Brandes, D.*: Histochemical reactions for electron microscopy: Acid phosphatase. *Exptl. Cell Research.* 9, 592, 1955.
- Sjöstrand, F. S.*: An electron microscope study of the retinal rods of the guinea pig eye. *J. Cell Comp. Physiol.* 33, 383, 1949 a.
- Sjöstrand, F. S.*: On multiple membrane structures in the retinal rods of the guinea pig eye and in nerve tissue. *Proc. Delft Congr. Electron Microscopy 1949 b.*
- Sjöstrand, F. S.*: Electron microscopic demonstration of a membrane structure isolated from nerve tissue. *Nature.* 165, 482, 1950.
- Sjöstrand, F. S.*: The lamellated structure of the nerve myelin sheath as revealed by high resolution electron microscopy. *Experientia.* 9, 68, 1953 a.
- Sjöstrand, F. S.*: A new microtome for ultrathin sectioning for high resolution electron microscopy. *Experientia.* 9, 114, 1953 b.
- Sjöstrand, F. S.*: The ultrastructure of the retinal rod synapses of the guinea pig eye. *J. Appl. Phys.* 24, 1422, 1953 c.

- Sjöstrand, F. S.*: The ultrastructure of the outer segments of rods and cones of the eye as revealed by the electron microscope. *J. Cell Comp. Physiol.* 42, 15, 1953 d.
- Sjöstrand, F. S.*: The ultrastructure of the inner segments of the retinal rods of the guinea-pig eye as revealed by electron microscopy. *J. Cell. Comp. Physiol.* 42, 1953 e.
- Sjöstrand, F. S.*: Electron microscopy of mitochondria and cytoplasmic double membranes. *Nature.* 171, 30, 1953 f.
- Sjöstrand, F. S.*: Die routinemässige Herstellung von ultradünnen (ca 200 Å). Gewebeschnitten für elektronenmikroskopische Untersuchungen der Gewebszellen bei hoher Auflösung. *Z. wiss. Mikroskop.* 62, 65, 1954 a.
- Sjöstrand, F. S.*: Recent development of electron microscopy as applied to the study of tissue cell ultrastructure. Rapport Europ. Congr. Electron Microscop. Gent. 69, 1954 b.
- Sjöstrand, F. S.*: Fine structure of cells. Symp. VIIIth Congr. Cell Biol., Leiden 1954. Internat. Union Biol. Sci. Series B — Nr 21, Noordhoff Ltd, 16, 1955 a.
- Sjöstrand, F. S.*: Fine structure of cells. Symp. VIIIth Congr. Cell Biol. Leiden 1954. Internat. Union Biol. Sci. Series B — Nr 21, Noordhoff Ltd, 222, 1955 b.
- Sjöstrand, F. S.*: Étapes du développement de la méthode de préparation des coupes de tissus ultrafines pour la microscopie électronique à haute résolution. Compt. rend. Colloque Intern. Tech. Rec. Microscopie. Electronique Corpusc. Toulouse. 151, 1955 c.
- Sjöstrand, F. S.*: A method to improve contrast in high resolution electron microscopy of ultrathin tissue sections. *Exptl. Cell Research.* 10, 657, 1956 a.
- Sjöstrand, F. S.*: The ultrastructure of cells as revealed by the electron microscope. Intern. Review. Cytol. 5, 455, 1956 b.
- Sjöstrand, F. S.*: Electron microscopy of cells and tissues. Psysical Techniques in Biol. Research. Academic Press, New York. Vol. III, 241, 1956 c.
- Sjöstrand, F. S. and Andersson, E.*: Electron microscopy of the intercalated discs of cardiac muscle tissue. *Experientia.* 10, 369, 1954.
- Sjöstrand, F. S. and Hanzon, V.*: Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy. *Exptl. Cell Research.* 7, 393, 1954 a.
- Sjöstrand, F. S. and Hanzon, V.*: Electron microscopy of the Golgi apparatus of the exocrine pancreas cells. *Experientia.* 10, 367, 1954 b.
- Sjöstrand, F. S. and Hanzon, V.*: Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas. *Exptl. Cell Research.* 7, 415, 1954 c.
- Sjöstrand, F. S. and Rhodin, J.*: The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. *Exptl. Cell Research.* 4, 426, 1953.
- Steinmann, E. and Sjöstrand, F. S.*: The ultrastructure of chloroplast. *Exptl. Cell Research.* 8, 15, 1955.

- Watson, M. L.: Pores in the mammalian nuclear membrane. *Biochem. Biophys. Acta.* 15, 475, 1954.
- Watson, M. L.: The nuclear envelope. Its structure and relation to cytoplasmic membranes. *J. Biophys. Biochem. Cytol.* 1, 257, 1955.
- Weinstein, P. and Forgács, J.: Data concerning the mechanism of the eye tension lowering effect of Diamox. *Ophthalmologica.* 130, 159, 1955.
- Weiss, J. M.: The ergostoplasm. Its fine structure and relation to protein synthesis as studied with the electron microscope in the pancreas of the Swiss albino mouse. *J. Exptl. Med.* 98, 607, 1953.
- Wersäll, J.: The minute structure of the crista ampullaris in the guinea pig as revealed by the electron microscope. *Acta Oto-Laryngol.* 44, 359, 1954.
- Wersäll, J.: Studies on the structure and innervation of the sensory epithelium of the cristae ampullaris in the guinea pig. Stockholm 1956.
- Williams, R. C. and Kallman, F.: Interpretations of electron micrographs of single and serial sections. *J. Biophys. Biochem. Cytol.* 1, 301, 1955.
- Wistrand, P. J.: Carbonic anhydrase in the anterior uvea of the rabbit. *Acta Physiol. Scand.* 24, 144, 1951.
- Wistrand, P. J.: Local action of a carbonic anhydrase inhibitor, acetazoleamide, on the intraocular pressure in cats. *Acta pharmacol. toxicol.* In press.
- Wolff, E.: The anatomy of the eye and orbit. London 1948.
- Yamada, E.: The fine structure of the renal glomerulus of the mouse. *J. Histochem. Cytochem.* 3, 309, 1955.
- Zetterqvist, H.: The ultrastructural organization of the columnar absorbing cells of the mouse jejunum. Stockholm 1956.
- Zetterqvist, H. and Sheldon, H.: Internal ultrastructure in granules of white blood cells of the mouse. *Bull. Johns Hopkins Hosp.* 96, 135, 1955.

